Introduction to RStudio Server

The CCDL

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

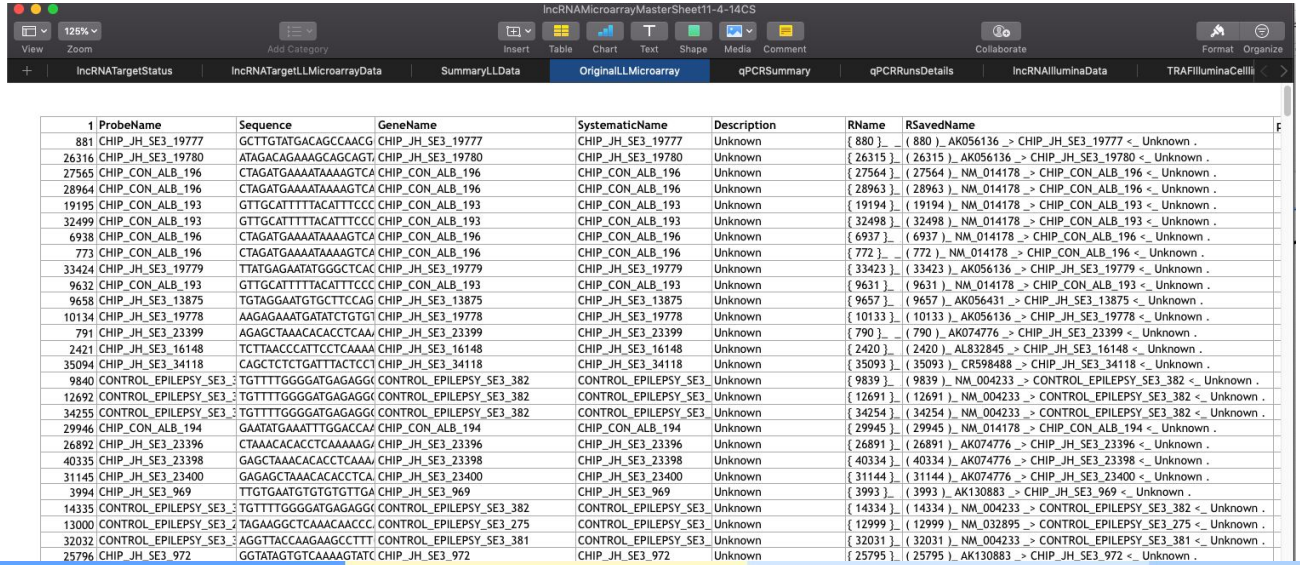
Differential Analysis Example 1: "100% Up to Date Analysis"

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.

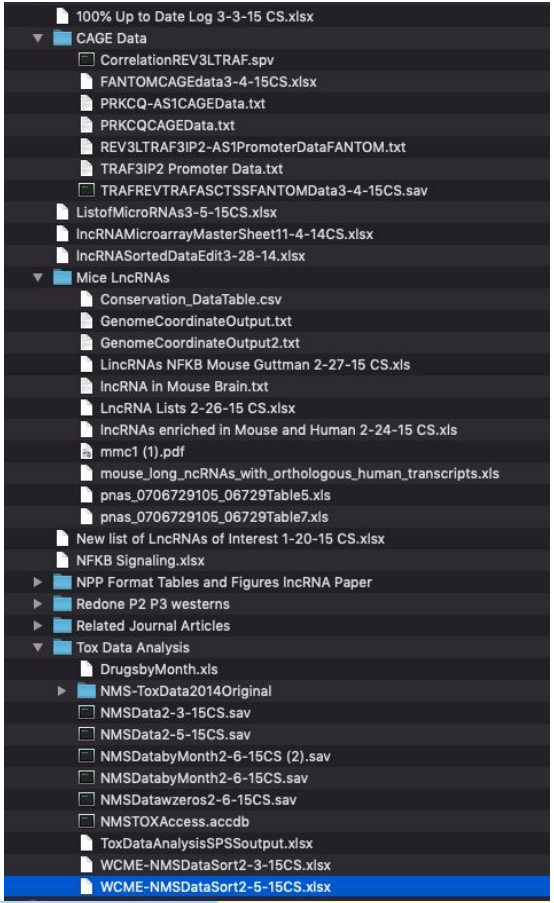
Data availability: The original data are on a flashdrive, in a desk drawer, in a lab. The already processed version is on GEO.

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

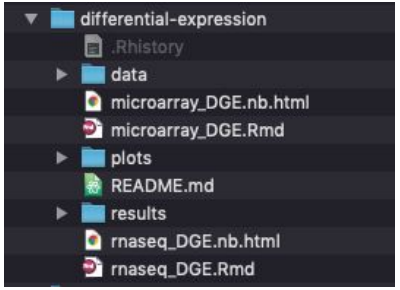
Software versions: No idea what software package(s), regardless of version.



1	ProbeName	Sequence	GeneName	SystematicName	Description	RName	RSavedName
881	CHIP_JH_SE3_19777	GCTTGTATGACAGCCAACG	CHIP_JH_SE3_19777	CHIP_JH_SE3_19777	Unknown	{ 880 }_ _	(880)_ AK056136 _> CHIP_JH_SE3_19777 <_ Unknown .
26316	CHIP_JH_SE3_19780	ATAGACAGAAAGCAGCAGT/	CHIP_JH_SE3_19780	CHIP_JH_SE3_19780	Unknown	{ 26315 }_	(26315)_ AK056136 _> CHIP_JH_SE3_19780 <_ Unknown .
27565	CHIP_CON_ALB_196	CTAGATGAAAATAAAAGTCA	CHIP_CON_ALB_196	CHIP_CON_ALB_196	Unknown	{ 27564 }_	(27564)_ NM_014178 _> CHIP_CON_ALB_196 <_ Unknown .
28964	CHIP_CON_ALB_196	CTAGATGAAAATAAAAGTCA	CHIP_CON_ALB_196	CHIP_CON_ALB_196	Unknown	{ 28963 }_	(28963)_ NM_014178 _> CHIP_CON_ALB_196 <_ Unknown .
19195	CHIP_CON_ALB_193	GTTGCATTTTTACATTTCCC	CHIP_CON_ALB_193	CHIP_CON_ALB_193	Unknown	{ 19194 }_	(19194)_ NM_014178 _> CHIP_CON_ALB_193 <_ Unknown .
32499	CHIP_CON_ALB_193	GTTGCATTTTTACATTTCCC	CHIP_CON_ALB_193	CHIP_CON_ALB_193	Unknown	{ 32498 }_	(32498)_ NM_014178 _> CHIP_CON_ALB_193 <_ Unknown .
6938	CHIP_CON_ALB_196	CTAGATGAAAATAAAAGTCA	CHIP_CON_ALB_196	CHIP_CON_ALB_196	Unknown	{ 6937 }_	(6937)_ NM_014178 _> CHIP_CON_ALB_196 <_ Unknown .
773	CHIP_CON_ALB_196	CTAGATGAAAATAAAAGTCA	CHIP_CON_ALB_196	CHIP_CON_ALB_196	Unknown	{ 772 }_ _	(772)_ NM_014178 _> CHIP_CON_ALB_196 <_ Unknown .
33424	CHIP_JH_SE3_19779	TTATGAGAATATGGGCTCAC	CHIP_JH_SE3_19779	CHIP_JH_SE3_19779	Unknown	{ 33423 }_	(33423)_ AK056136 _> CHIP_JH_SE3_19779 <_ Unknown .
9632	CHIP_CON_ALB_193	GTTGCATTTTTACATTTCCC	CHIP_CON_ALB_193	CHIP_CON_ALB_193	Unknown	{ 9631 }_	(9631)_ NM_014178 _> CHIP_CON_ALB_193 <_ Unknown .
9658	CHIP_JH_SE3_13875	TGTAGGAATGTGCTTCCAG	CHIP_JH_SE3_13875	CHIP_JH_SE3_13875	Unknown	{ 9657 }_	(9657)_ AK056431 _> CHIP_JH_SE3_13875 <_ Unknown .
10134	CHIP_JH_SE3_19778	AAGAGAAATGATATCTGTGT	CHIP_JH_SE3_19778	CHIP_JH_SE3_19778	Unknown	{ 10133 }_	(10133)_ AK056136 _> CHIP_JH_SE3_19778 <_ Unknown .
791	CHIP_JH_SE3_23399	AGAGCTAAACACACCTCAA/	CHIP_JH_SE3_23399	CHIP_JH_SE3_23399	Unknown	{ 790 }_ _	(790)_ AK074776 _> CHIP_JH_SE3_23399 <_ Unknown .
2421	CHIP_JH_SE3_16148	TCTTAACCCATTCCTCAAAA	CHIP_JH_SE3_16148	CHIP_JH_SE3_16148	Unknown	{ 2420 }_	(2420)_ AL832845 _> CHIP_JH_SE3_16148 <_ Unknown .
35094	CHIP_JH_SE3_34118	CAGCTCTCTGATTTACTCCT	CHIP_JH_SE3_34118	CHIP_JH_SE3_34118	Unknown	{ 35093 }_	(35093)_ CR598488 _> CHIP_JH_SE3_34118 <_ Unknown .
9840	CONTROL_EPILEPSY_SE3_3	TGTTTTGGGGATGAGAGG(	CONTROL_EPILEPSY_SE3_382	CONTROL_EPILEPSY_SE3_	Unknown	{ 9839 }_	(9839)_ NM_004233 _> CONTROL_EPILEPSY_SE3_382 <_ Unknown .
12692	CONTROL_EPILEPSY_SE3_3	TGTTTTGGGGATGAGAGG(	CONTROL_EPILEPSY_SE3_382	CONTROL_EPILEPSY_SE3_	Unknown	{ 12691 }_	(12691)_ NM_004233 _> CONTROL_EPILEPSY_SE3_382 <_ Unknown .
34255	CONTROL_EPILEPSY_SE3_3	TGTTTTGGGGATGAGAGG(	CONTROL_EPILEPSY_SE3_382	CONTROL_EPILEPSY_SE3_	Unknown	{ 34254 }_	(34254)_ NM_004233 _> CONTROL_EPILEPSY_SE3_382 <_ Unknown .
29946	CHIP_CON_ALB_194	GAATATGAAATTTGGACCA/	CHIP_CON_ALB_194	CHIP_CON_ALB_194	Unknown	{ 29945 }_	(29945)_ NM_014178 _> CHIP_CON_ALB_194 <_ Unknown .
26892	CHIP_JH_SE3_23396	CTAAACACACCTCAAAAAG/	CHIP_JH_SE3_23396	CHIP_JH_SE3_23396	Unknown	{ 26891 }_	(26891)_ AK074776 _> CHIP_JH_SE3_23396 <_ Unknown .
40335	CHIP_JH_SE3_23398	GAGCTAAACACACCTCAAA/	CHIP_JH_SE3_23398	CHIP_JH_SE3_23398	Unknown	{ 40334 }_	(40334)_ AK074776 _> CHIP_JH_SE3_23398 <_ Unknown .
31145	CHIP_JH_SE3_23400	GAGAGCTAAACACACCTCA/	CHIP_JH_SE3_23400	CHIP_JH_SE3_23400	Unknown	{ 31144 }_	(31144)_ AK074776 _> CHIP_JH_SE3_23400 <_ Unknown .
3994	CHIP_JH_SE3_969	TTGTGAATGTGTGTGTTGA	CHIP_JH_SE3_969	CHIP_JH_SE3_969	Unknown	{ 3993 }_	(3993)_ AK130883 _> CHIP_JH_SE3_969 <_ Unknown .
14335	CONTROL_EPILEPSY_SE3_3	TGTTTTGGGGATGAGAGG(	CONTROL_EPILEPSY_SE3_382	CONTROL_EPILEPSY_SE3_	Unknown	{ 14334 }_	(14334)_ NM_004233 _> CONTROL_EPILEPSY_SE3_382 <_ Unknown .
13000	CONTROL_EPILEPSY_SE3_2	TAGAAGGCTCAAACAACCC	CONTROL_EPILEPSY_SE3_275	CONTROL_EPILEPSY_SE3_	Unknown	{ 12999 }_	(12999)_ NM_032895 _> CONTROL_EPILEPSY_SE3_275 <_ Unknown .
32032	CONTROL_EPILEPSY_SE3_3	AGGTTACCAAGAAGCCTTT	CONTROL_EPILEPSY_SE3_381	CONTROL_EPILEPSY_SE3_	Unknown	{ 32031 }_	(32031)_ NM_004233 _> CONTROL_EPILEPSY_SE3_381 <_ Unknown .
25796	CHIP_JH_SE3_972	GGTATAGTGTCAAAAGTATC	CHIP_JH_SE3_972	CHIP_JH_SE3_972	Unknown	{ 25795 }_	(25795)_ AK130883 _> CHIP_JH_SE3_972 <_ Unknown .



Data Analysis Example 2: "GitHubbed Analysis"

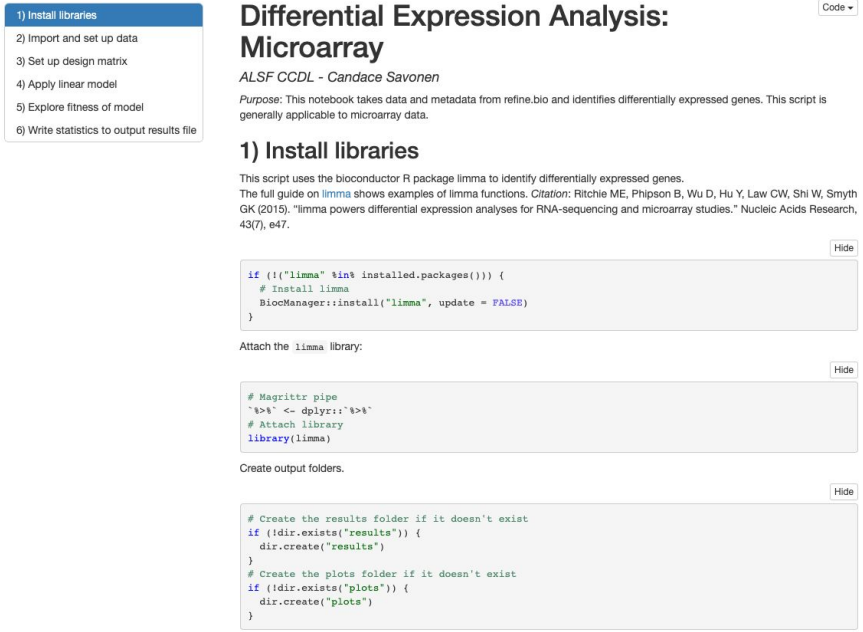


Methods Documentation: Publicly available, online notebooks show step-by-step process that can be re-run.

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

Code

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

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.

Hide

```
if (!("limma" %in% installed.packages())) {
  # Install limma
  BiocManager::install("limma", update = FALSE)
}
```

Attach the `limma` library:

Hide

```
# Magrittr pipe
`%>%` <- dplyr::`%>%`
# Attach library
library(limma)
```

Create output folders.

Hide

```
# Create the results folder if it doesn't exist
if (!dir.exists("results")) {
  dir.create("results")
}
# Create the plots folder if it doesn't exist
if (!dir.exists("plots")) {
  dir.create("plots")
}
```

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?

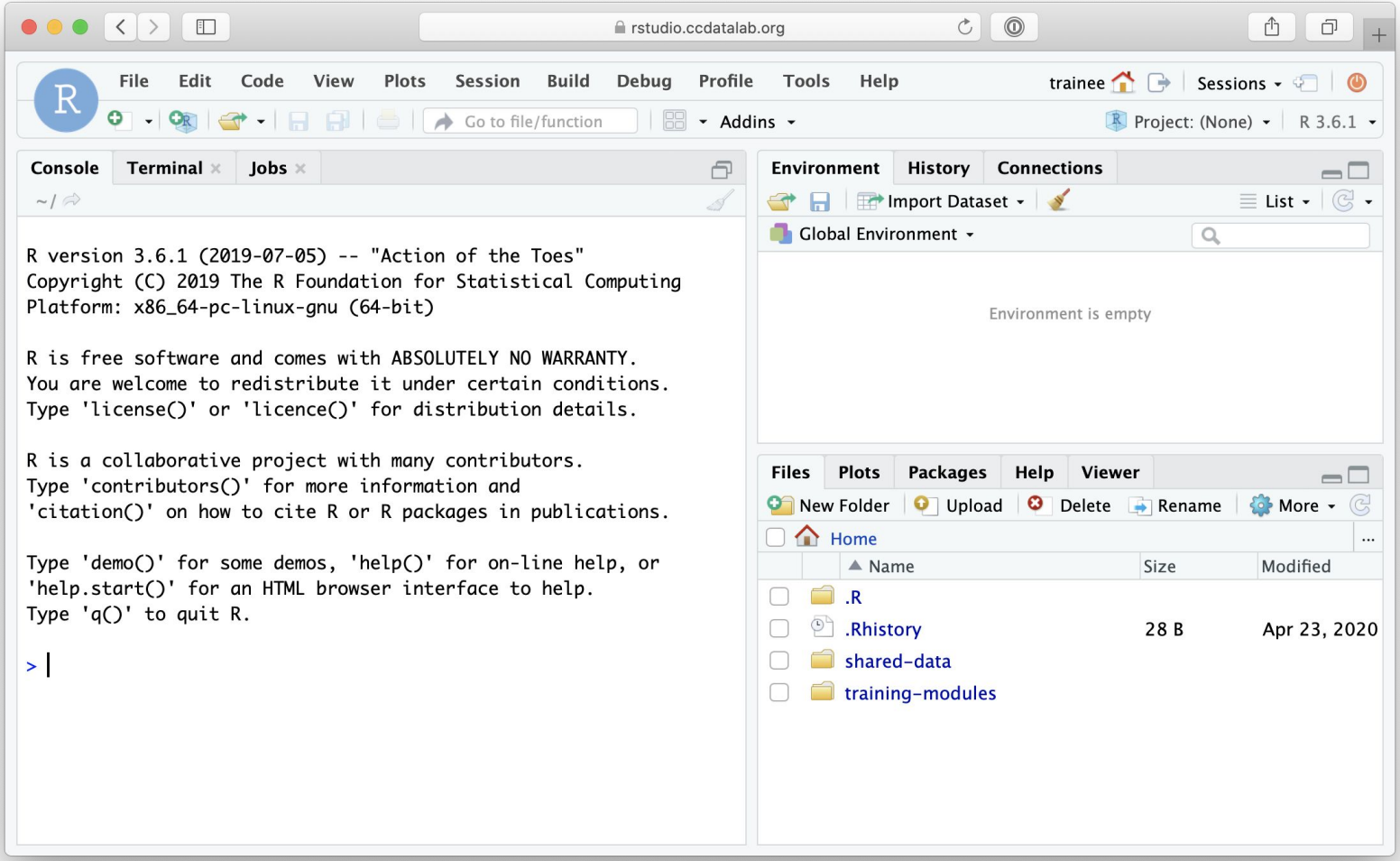
...feel would be *easier to reproduce*?

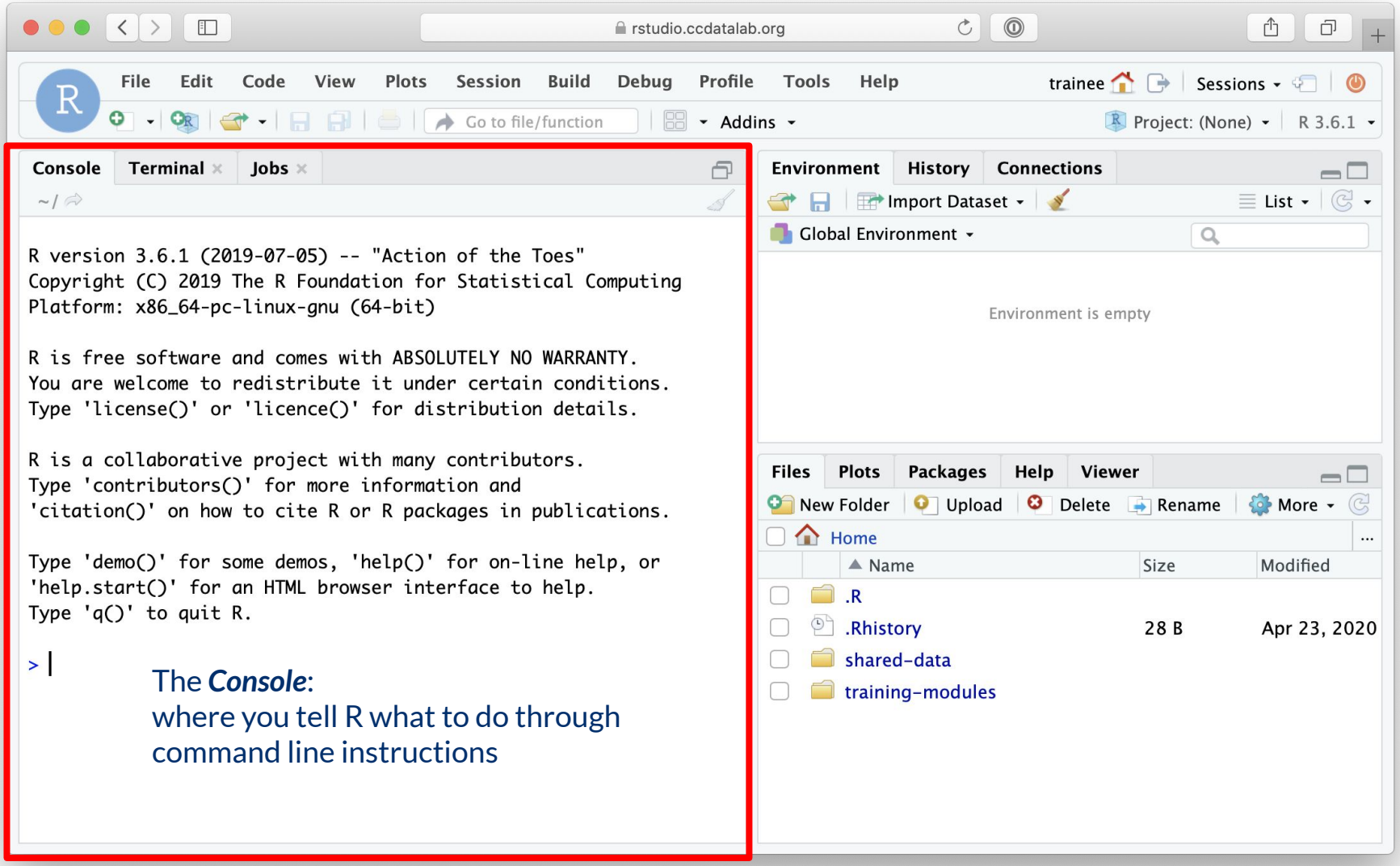
Command line vs GUI (graphics user interface)

- 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




rstudio.ccdatalab.org
File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
Console
Terminal
Jobs
~/
R version 3.6.1 (2019-07-05) -- "Action of the Toes"
Copyright (C) 2019 The R Foundation for Statistical Computing
Platform: x86_64-pc-linux-gnu (64-bit)
R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.
R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.
Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.
>
The Console:
where you tell R what to do through
command line instructions
Environment
History
Connections
Import Dataset
List
Global Environment
Environment is empty
Files
Plots
Packages
Help
Viewer
New Folder
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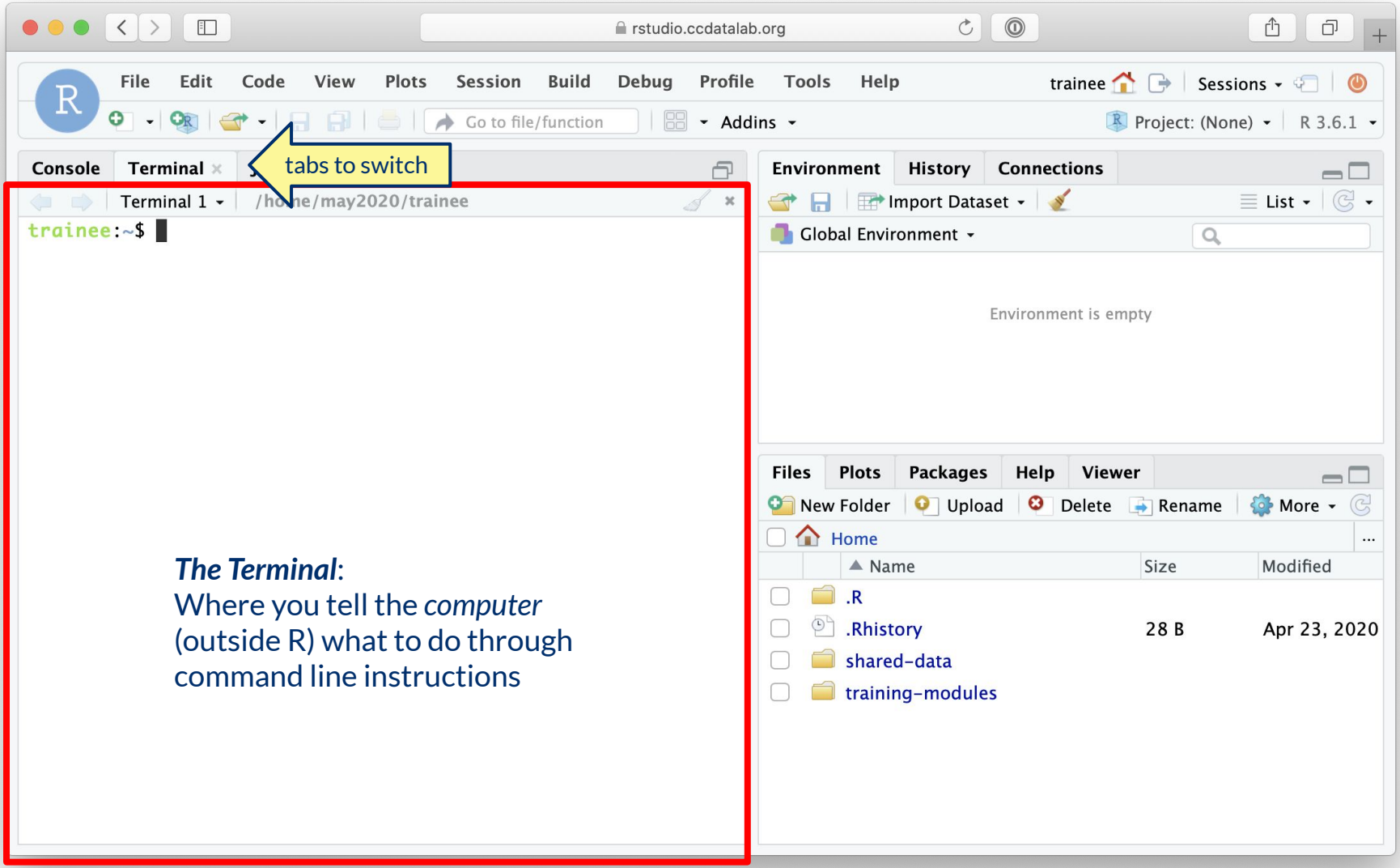
28 B
Apr 23, 2020
shared-data
training-modules


rstudio.ccdatalab.org
File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
Console
Terminal
tabs to switch
Terminal 1
/home/may2020/trainee
trainee:~$
The Terminal:
Where you tell the computer
(outside R) what to do through
command line instructions
Environment
History
Connections
Import Dataset
List
Global Environment
Environment is empty
Files
Plots
Packages
Help
Viewer
New Folder
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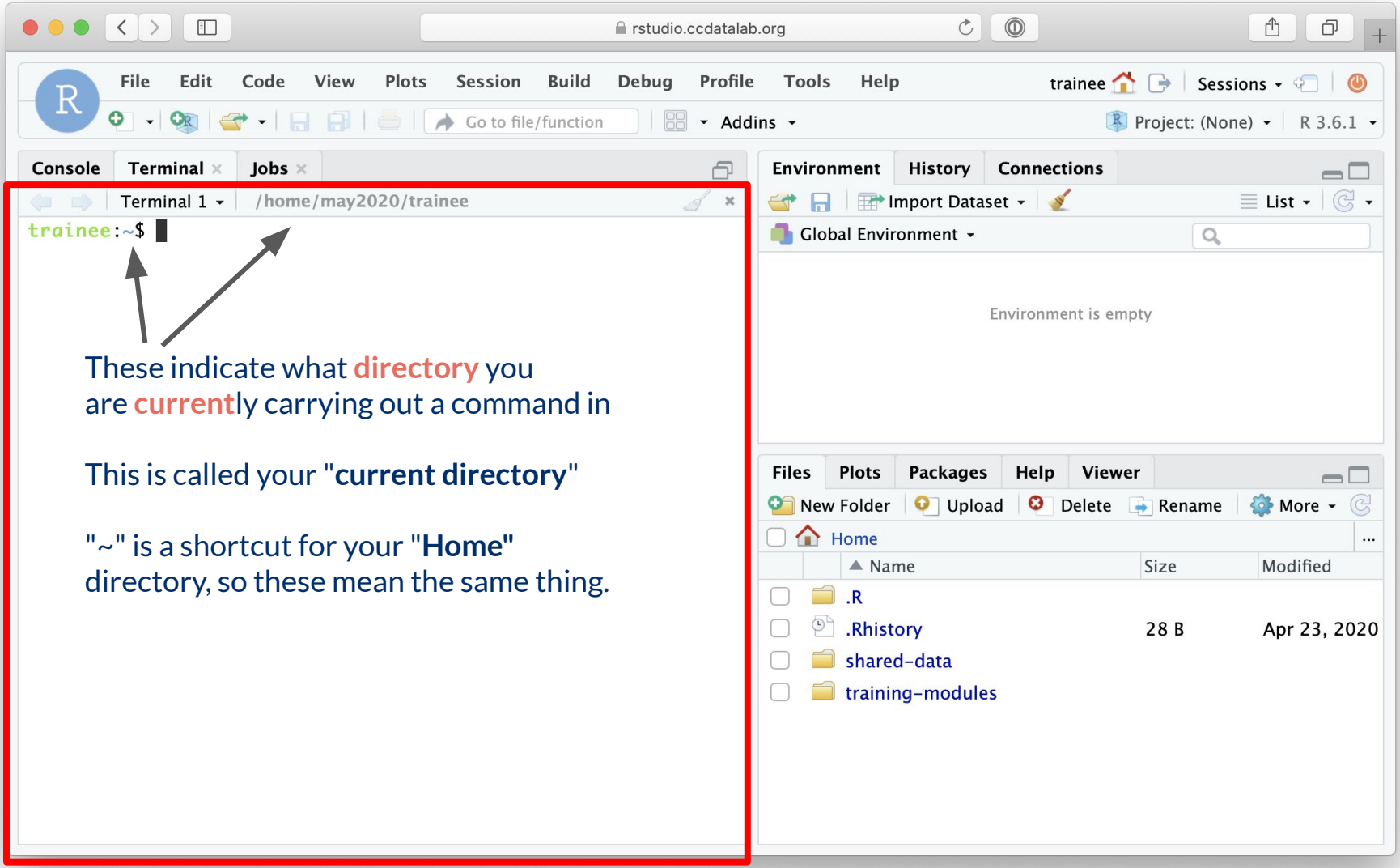
.Rhistory
28 B
Apr 23, 2020
shared-data
training-modules


rstudio.ccdatalab.org
File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
Console
Terminal
Jobs
Terminal 1
/home/may2020/trainee
trainee:~$
These indicate what directory you are currently carrying out a command in
This is called your "current directory"
"~" is a shortcut for your "Home" directory, so these mean the same thing.
Environment
History
Connections
Import Dataset
List
Global Environment
Environment is empty
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
Name
Size
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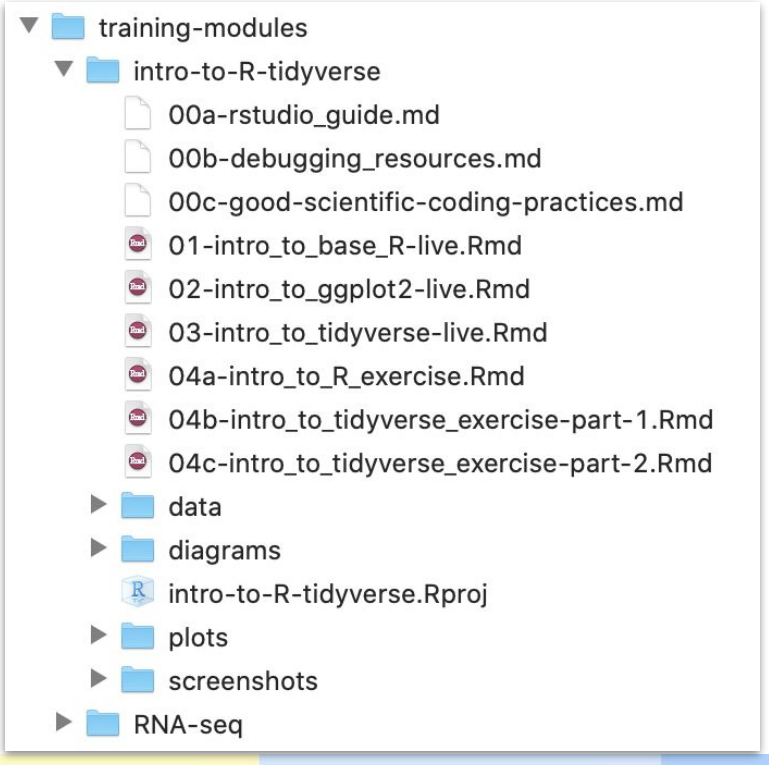
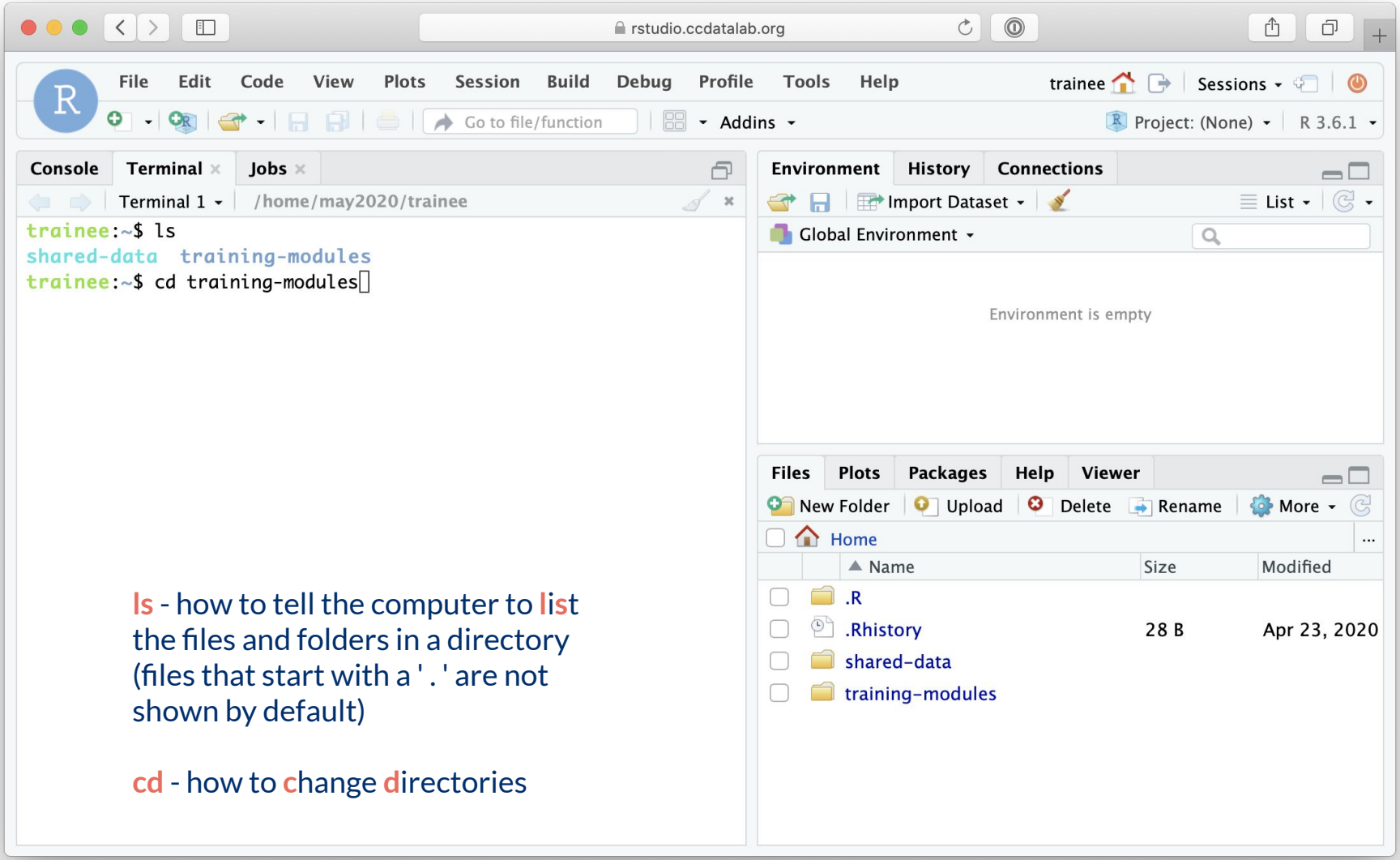
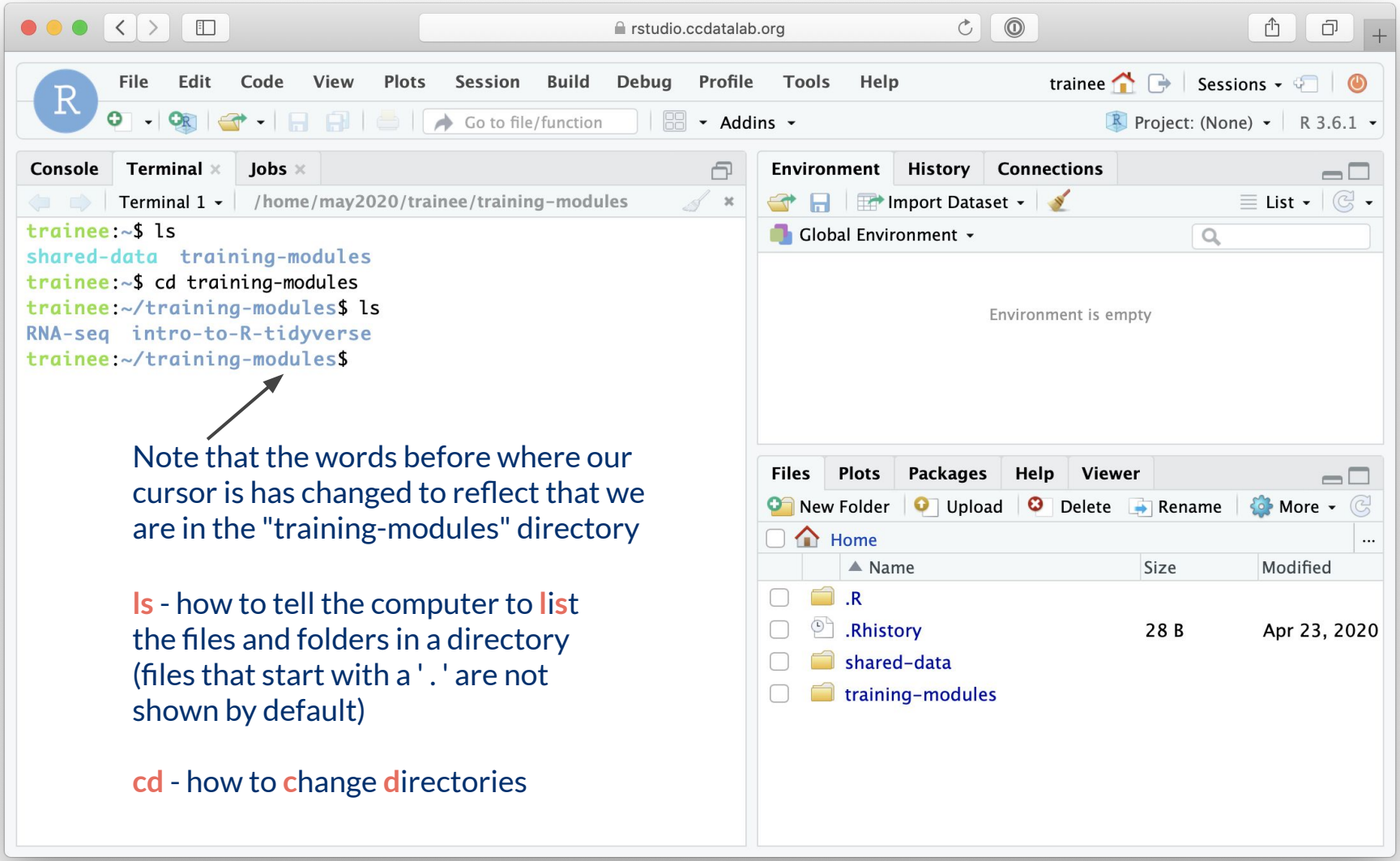
28 B
Apr 23, 2020
shared-data
training-modules

Directories = Folders

When we are doing things through command line, we have to look at where the files we are using are being kept.




rstudio.ccdatalab.org
File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
Console
Terminal
Jobs
Terminal 1
/home/may2020/trainee
trainee:~$ ls
shared-data training-modules
trainee:~$ cd training-modules
Environment
History
Connections
Import Dataset
List
Global Environment
Environment is empty
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
Name
Size
Modified
.R
.Rhistory
28 B
Apr 23, 2020
shared-data
training-modules
ls - how to tell the computer to list the files and folders in a directory (files that start with a ' . ' are not shown by default)
cd - how to change directories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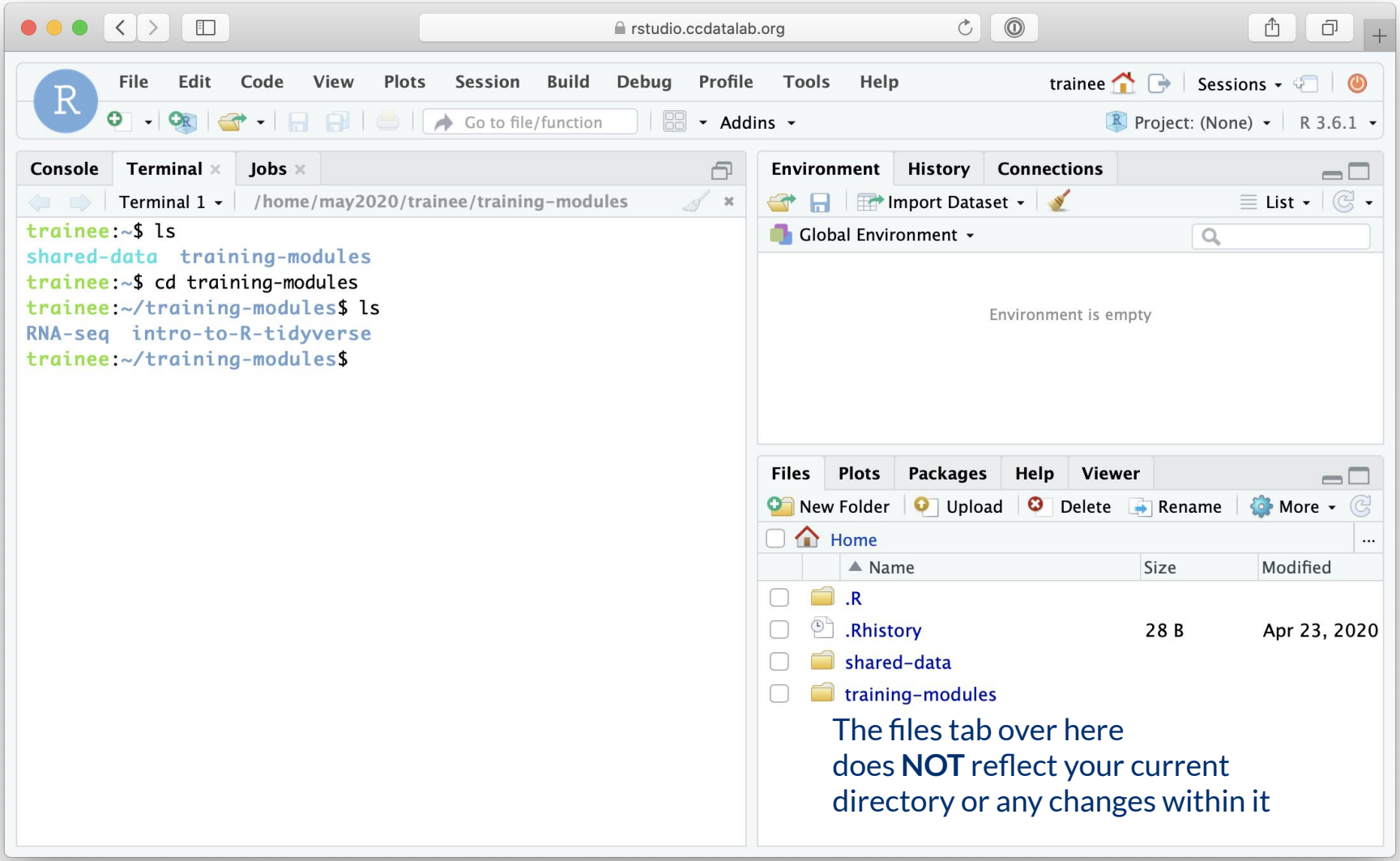
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
Console
Terminal
Jobs
Terminal 1
/home/may2020/trainee/training-modules
trainee:~$ ls
shared-data training-modules
trainee:~$ cd training-modules
trainee:~/training-modules$ ls
RNA-seq intro-to-R-tidyverse
trainee:~/training-modules$
Environment
History
Connections
Import Dataset
List
Global Environment
Environment is empty
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
Name
Size
Modified
.R
.Rhistory
28 B
Apr 23, 2020
shared-data
training-modules
Note that the words before where our cursor is has changed to reflect that we are in the "training-modules" directory
ls - how to tell the computer to list the files and folders in a directory (files that start with a ' . ' are not shown by default)
cd - how to change directories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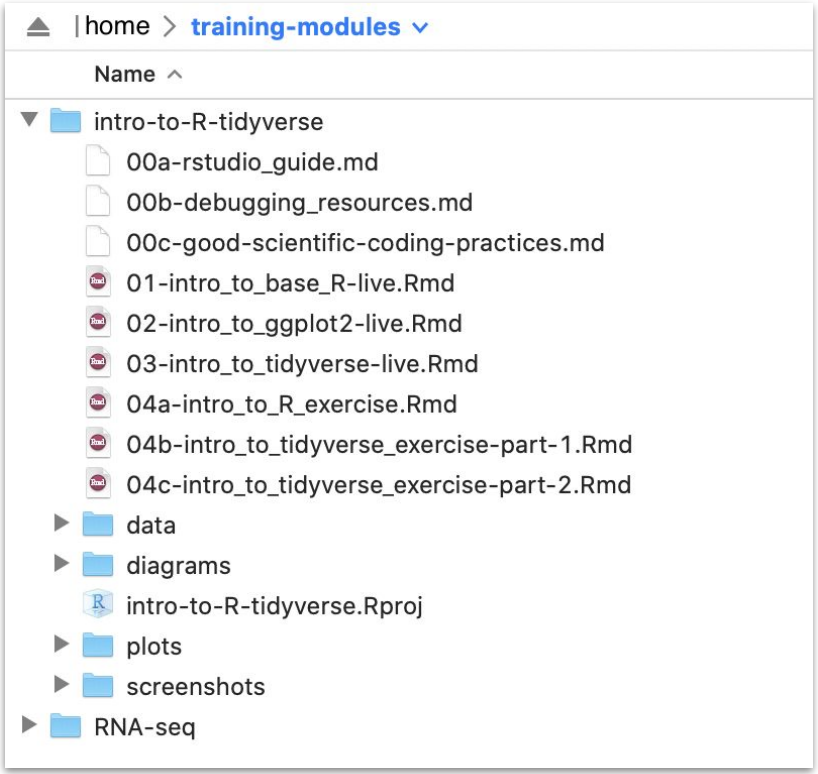
Addins
Project: (None)
R 3.6.1
Console
Terminal
Jobs
Terminal 1
/home/may2020/trainee/training-modules
trainee:~$ ls
shared-data training-modules
trainee:~$ cd training-modules
trainee:~/training-modules$ ls
RNA-seq intro-to-R-tidyverse
trainee:~/training-modules$
Environment
History
Connections
Import Dataset
List
Global Environment
Environment is empty
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
Name
Size
Modified
.R
.Rhistory
28 B
Apr 23, 2020
shared-data
training-modules
The files tab over here
does NOT reflect your current
directory or any changes within it

File paths: Directions to a file or folder

Let's say we want access to "01-intro_to_base_R-live.Rmd"

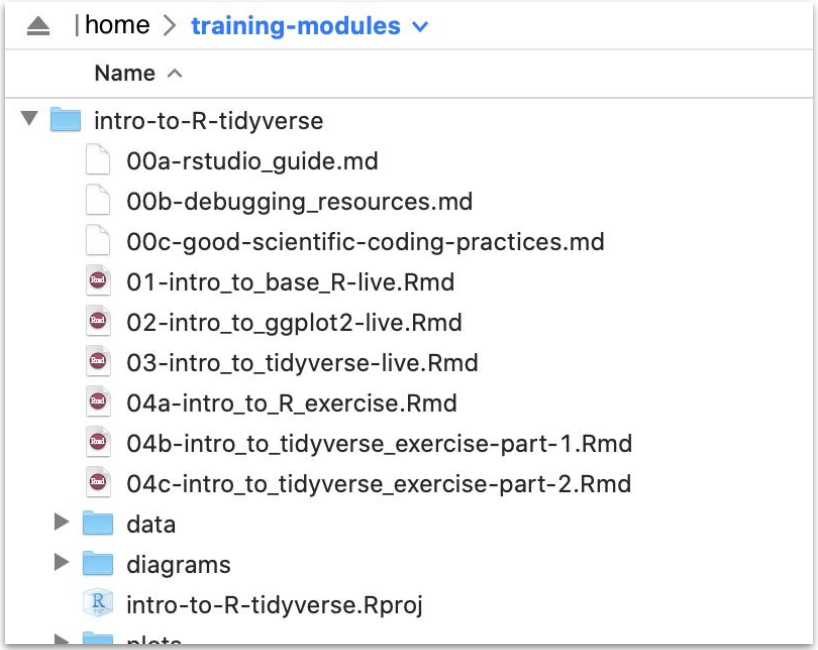


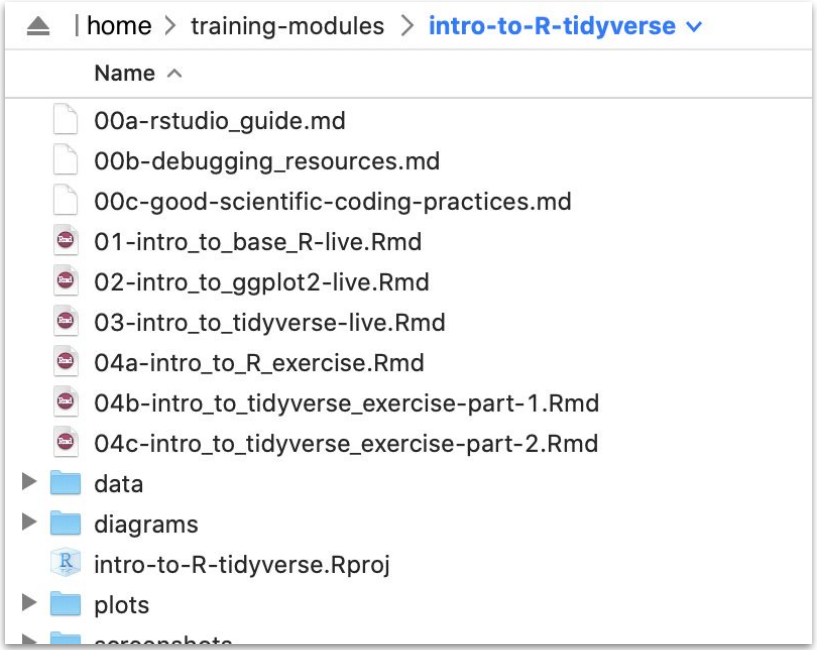
Current directory = "training-modules"

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"





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

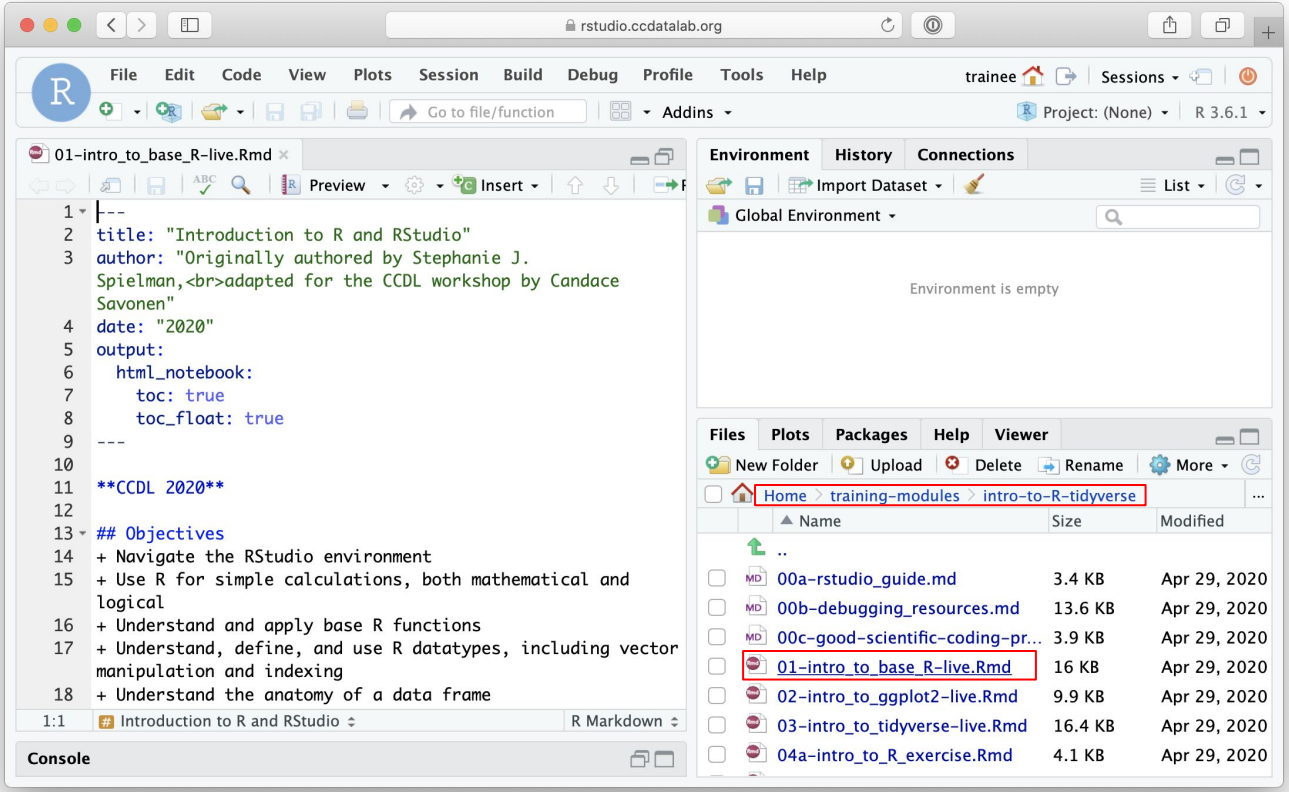
By Jessica Boddy | Aug. 29, 2016 , 1:45 PM

What you type	What you see	How Excel stores it
MARCH1	1-MAR	42430
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

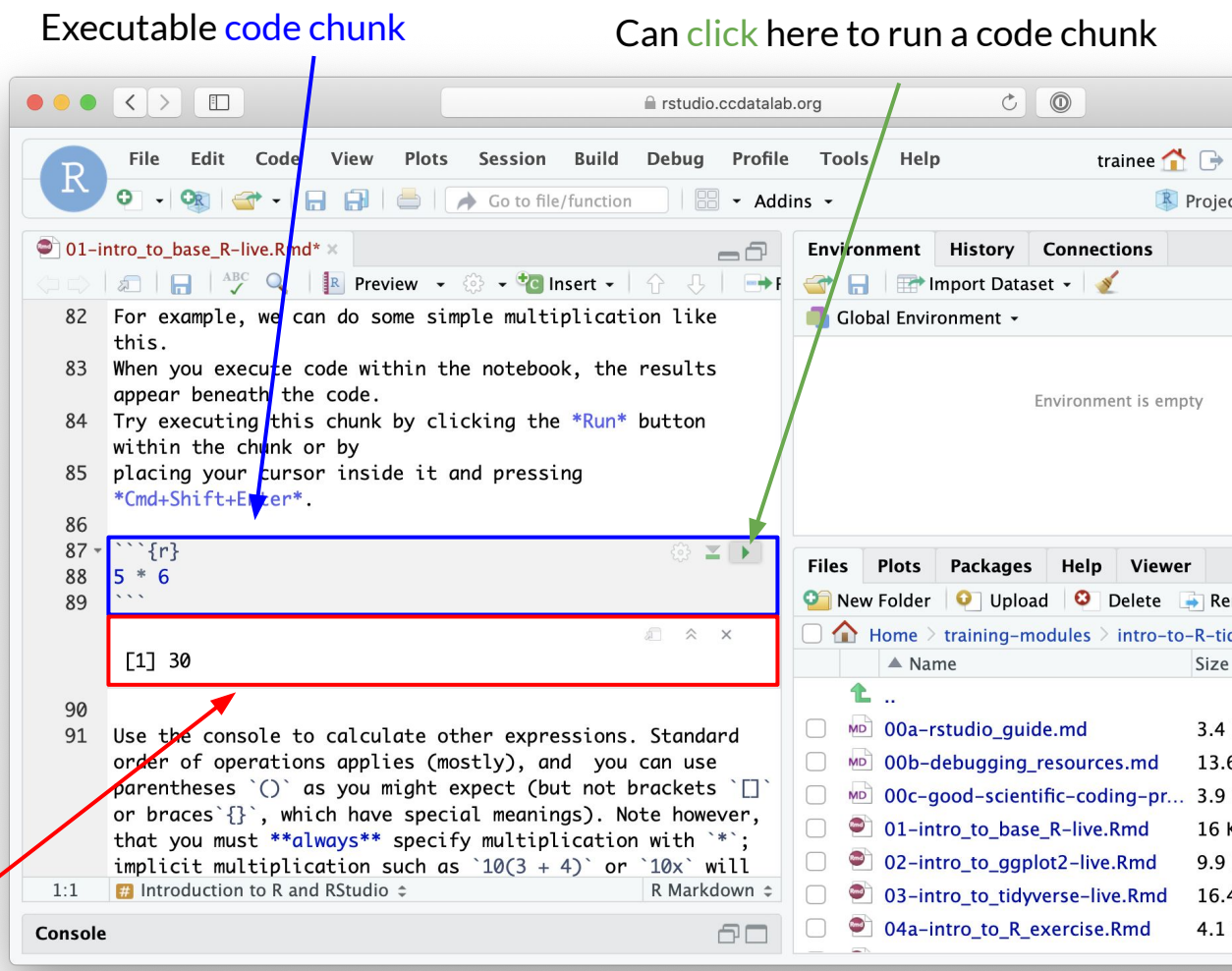
R Notebooks

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



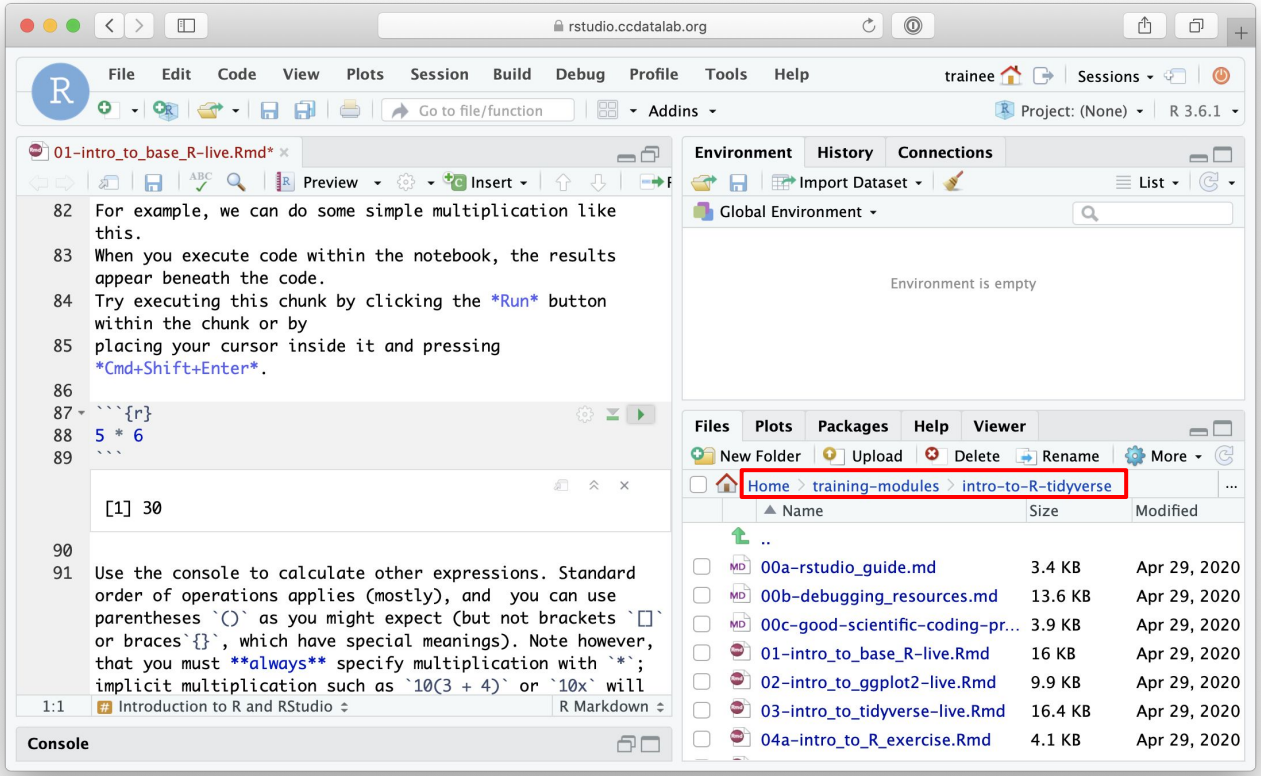
R Notebooks

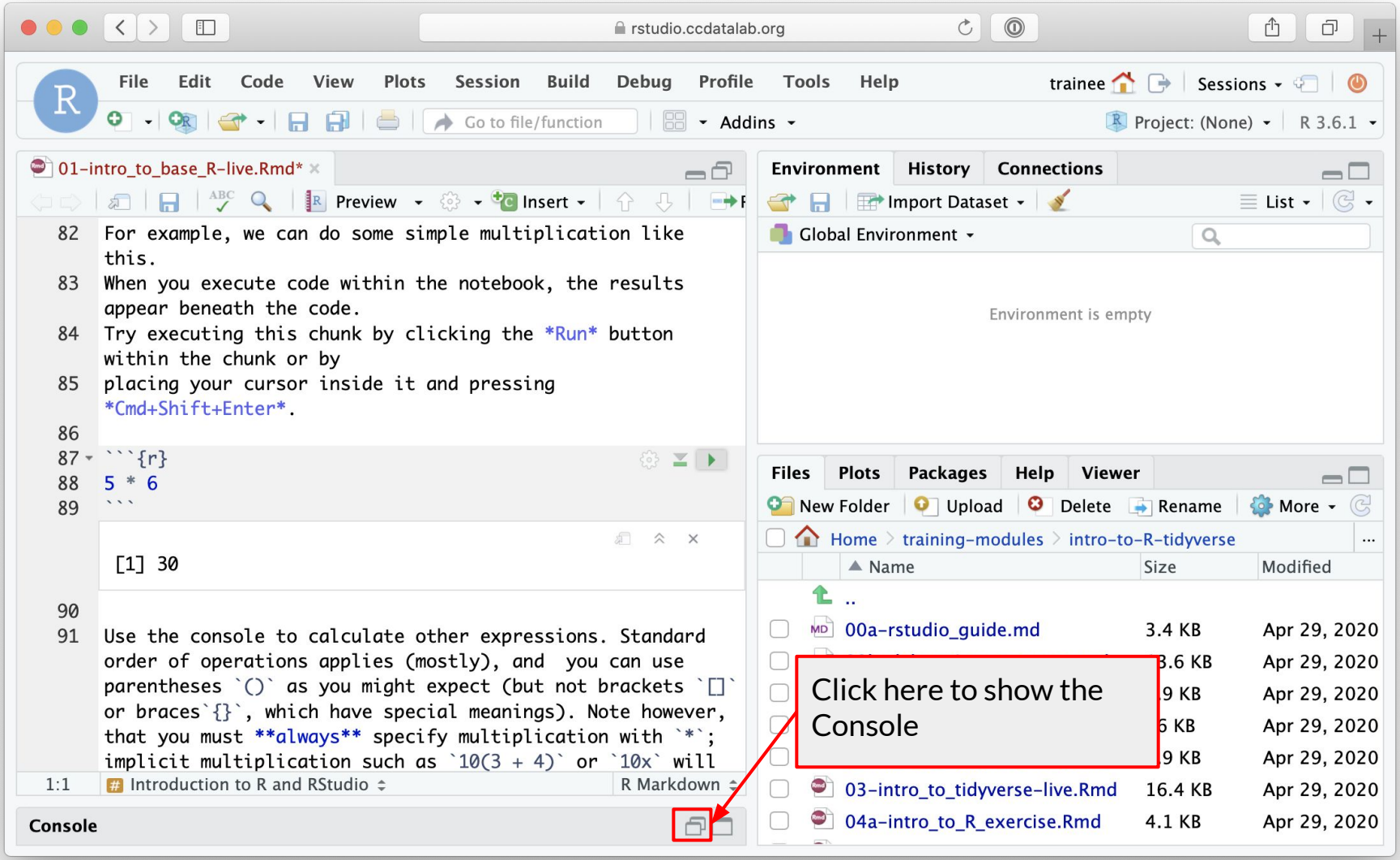
- R Notebooks allow you to have files that show both your code and results

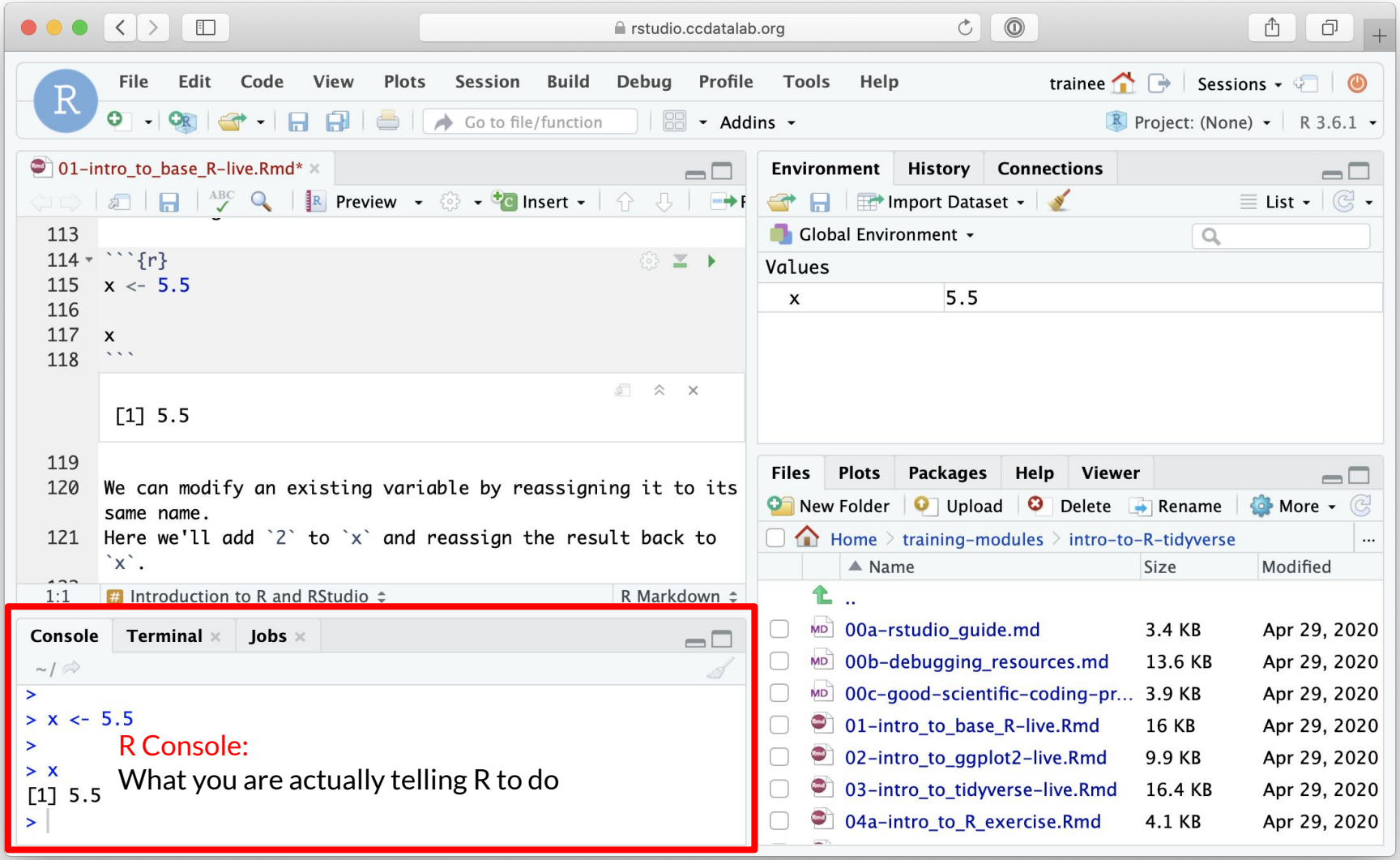


R Notebooks

- Code that runs in R Notebooks uses <u>wherever the file is saved</u> as its *current directory*




rstudio.ccdatalab.org
File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
01-intro_to_base_R-live.Rmd*
Preview
Insert
82 For example, we can do some simple multiplication like this.
83 When you execute code within the notebook, the results appear beneath the code.
84 Try executing this chunk by clicking the *Run* button within the chunk or by
85 placing your cursor inside it and pressing *Cmd+Shift+Enter*.
86
87 ```{r}
88 5 * 6
89 ```
[1] 30
90
91 Use the console to calculate other expressions. Standard order of operations applies (mostly), and you can use parentheses `()` as you might expect (but not brackets `[]` or braces`{}`, which have special meanings). Note however, that you must **always** specify multiplication with `*`; implicit multiplication such as `10(3 + 4)` or `10x` will
1:1
Introduction to R and RStudio
R Markdown
Console
Environment
History
Connections
Import Dataset
List
Global Environment
Environment is empty
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
training-modules
intro-to-R-tidyverse
Name
Size
Modified
..
00a-rstudio_guide.md 3.4 KB Apr 29, 2020
Apr 29, 2020
Apr 29, 2020
Apr 29, 2020
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
Click here to show the Console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File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
01-intro_to_base_R-live.Rmd*
Preview
Insert
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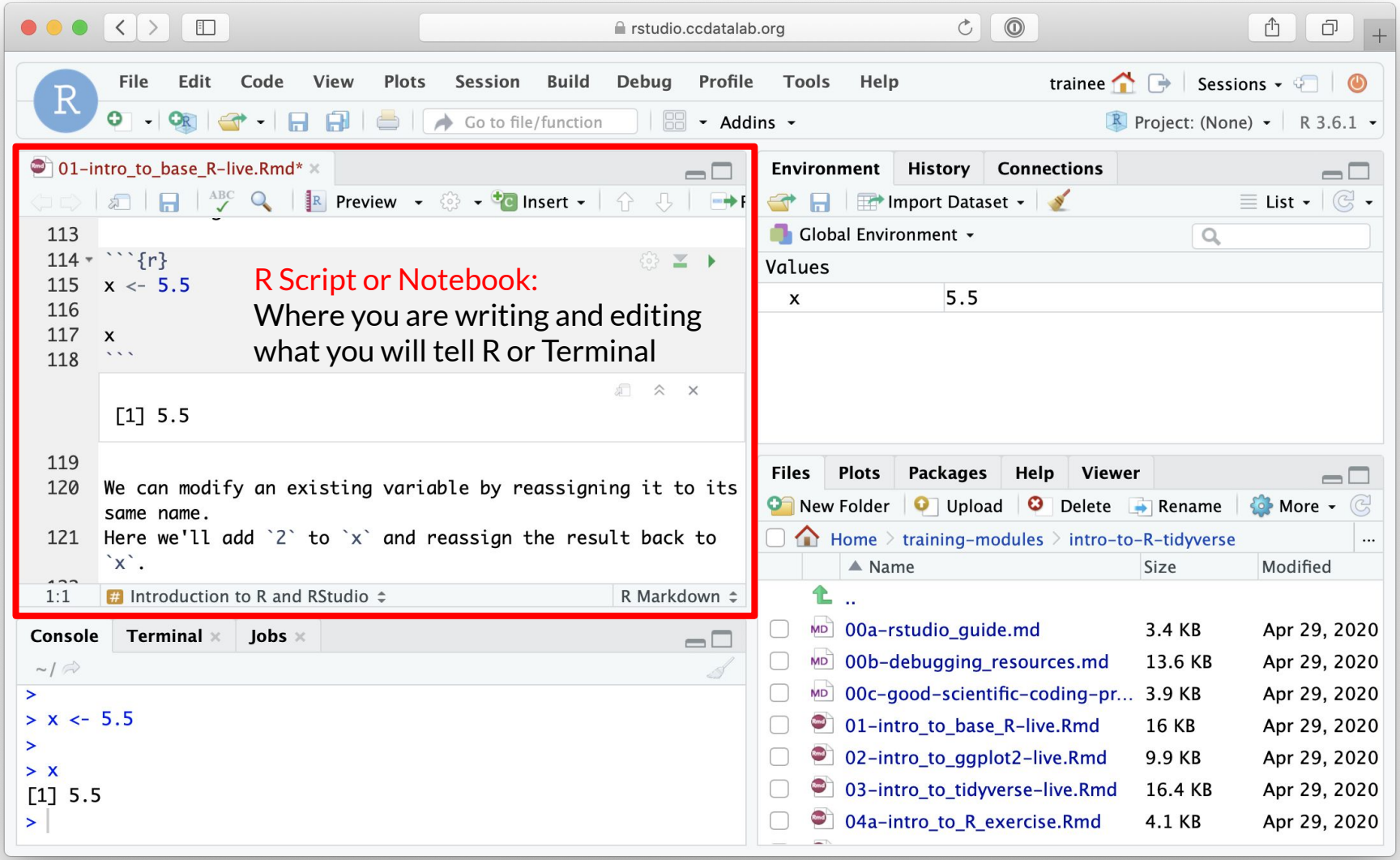
114 ```{r}
115 x <- 5.5
116
117 x
118 ```
[1] 5.5
119
120 We can modify an existing variable by reassigning it to its same name.
121 Here we'll add `2` to `x` and reassign the result back to `x`.
1:1
Introduction to R and RStudio
R Markdown
Environment
History
Connections
Import Dataset
List
Global Environment
Values
x 5.5
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
training-modules
intro-to-R-tidyverse
Name
Size
Modified
..
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
Console
Terminal
Jobs
~/
> x <- 5.5
> x
[1] 5.5
R Console:
What you are actually telling R to do


rstudio.ccdatalab.org
File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
Project: (None)
R 3.6.1
01-intro_to_base_R-live.Rmd*
Preview
Insert
113
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R Script or Notebook:
Where you are writing and editing what you will tell R or Terminal
1:1
Introduction to R and RStudio
R Markdown
Environment History Connections
Import Dataset
List
Global Environment
Values
x 5.5
Files Plots Packages Help Viewer
New Folder Upload Delete Rename More
Home training-modules intro-to-R-tidyverse
Name Size Modified
.. 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
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Console Terminal Jobs
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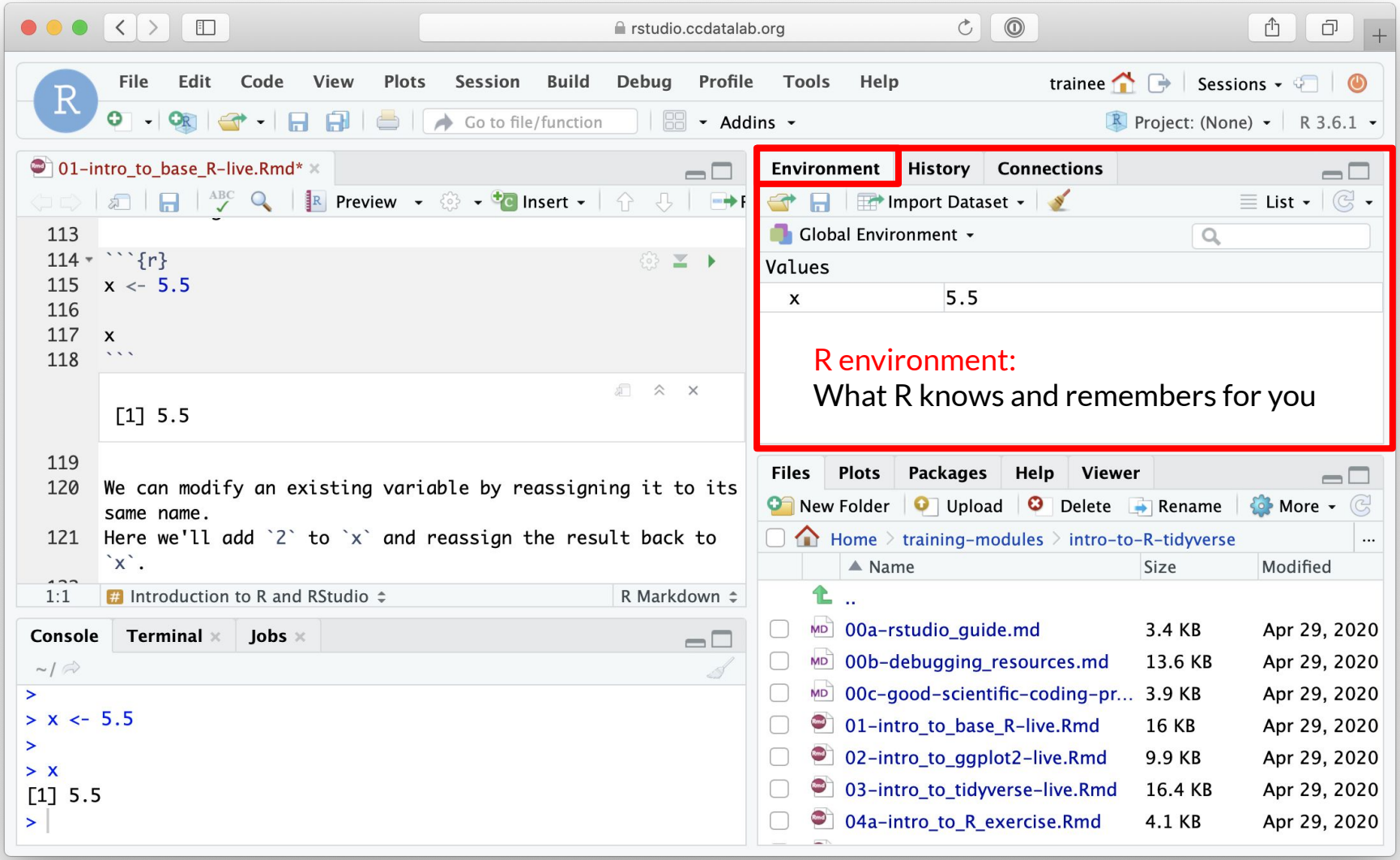
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File Edit Code View Plots Session Build Debug Profile Tools Help
trainee
Sessions
Go to file/function
Addins
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R 3.6.1
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Introduction to R and RStudio
R Markdown
Console
Terminal
Jobs
~/
> x <- 5.5
> x
[1] 5.5
Environment
History
Connections
Import Dataset
List
Global Environment
Values
x 5.5
R environment:
What R knows and remembers for you
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
training-modules
intro-to-R-tidyverse
Name
Size
Modified
..
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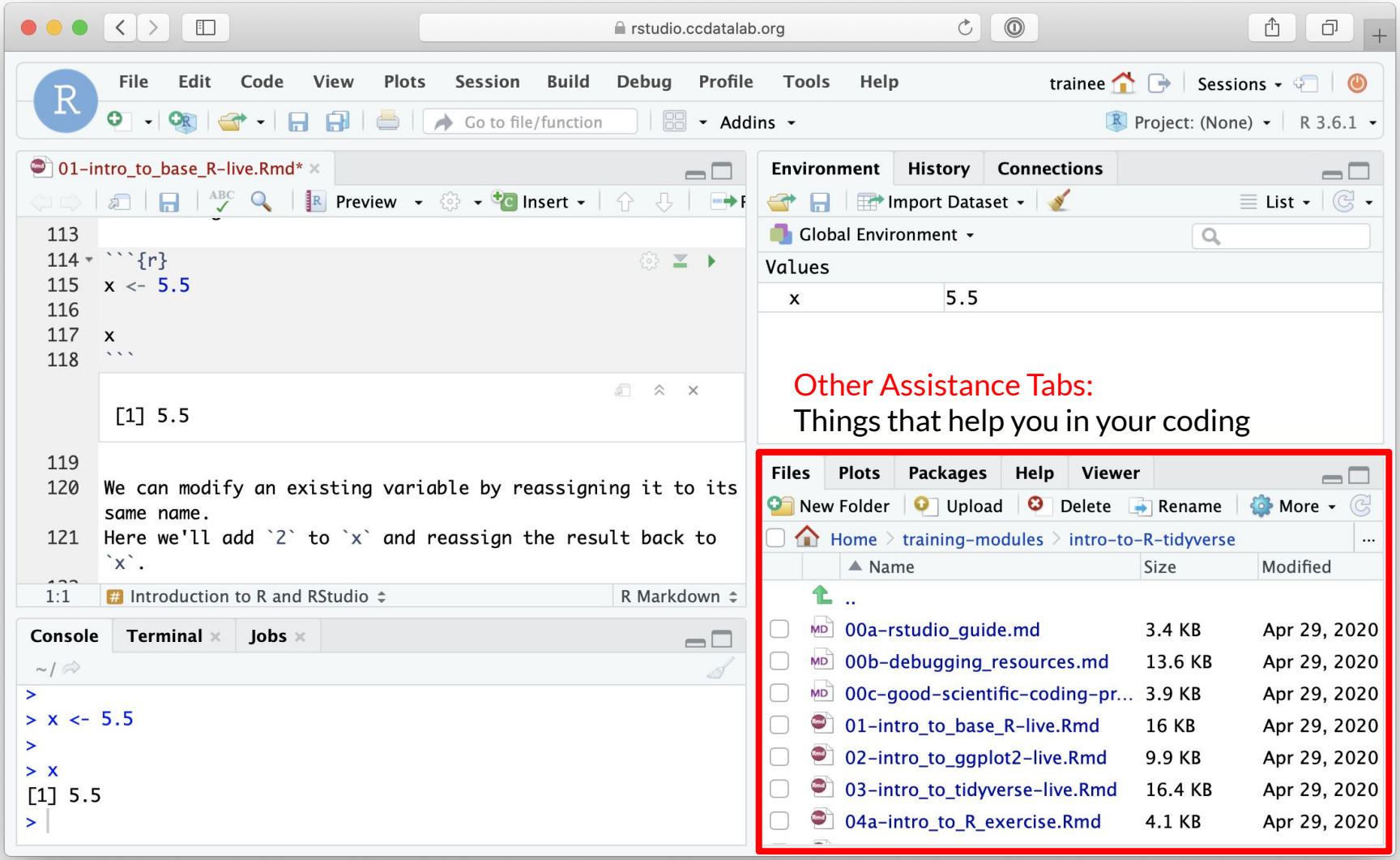
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Sessions
Go to file/function
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R Markdown
Console
Terminal
Jobs
~/
> x <- 5.5
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Environment
History
Connections
Import Dataset
List
Global Environment
Values
x 5.5
Other Assistance Tabs:
Things that help you in your coding
Files
Plots
Packages
Help
Viewer
New Folder
Upload
Delete
Rename
More
Home
training-modules
intro-to-R-tidyverse
Name
Size
Modified
..
00a-rstudio_guide.md 3.4 KB Apr 29, 2020
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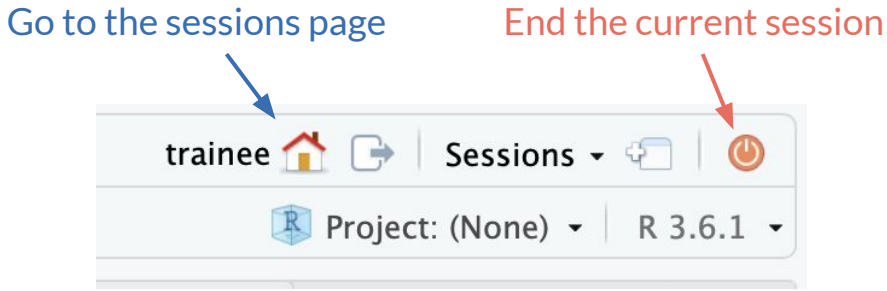
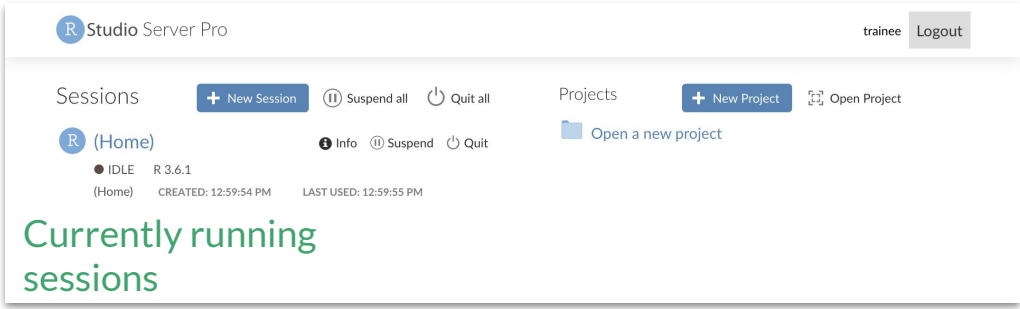
03-intro_to_tidyverse-live.Rmd 16.4 KB Apr 29, 2020
04a-intro_to_R_exercise.Rmd 4.1 KB Apr 29, 2020

RStudio Sessions

- On the server, R run 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



Session Page