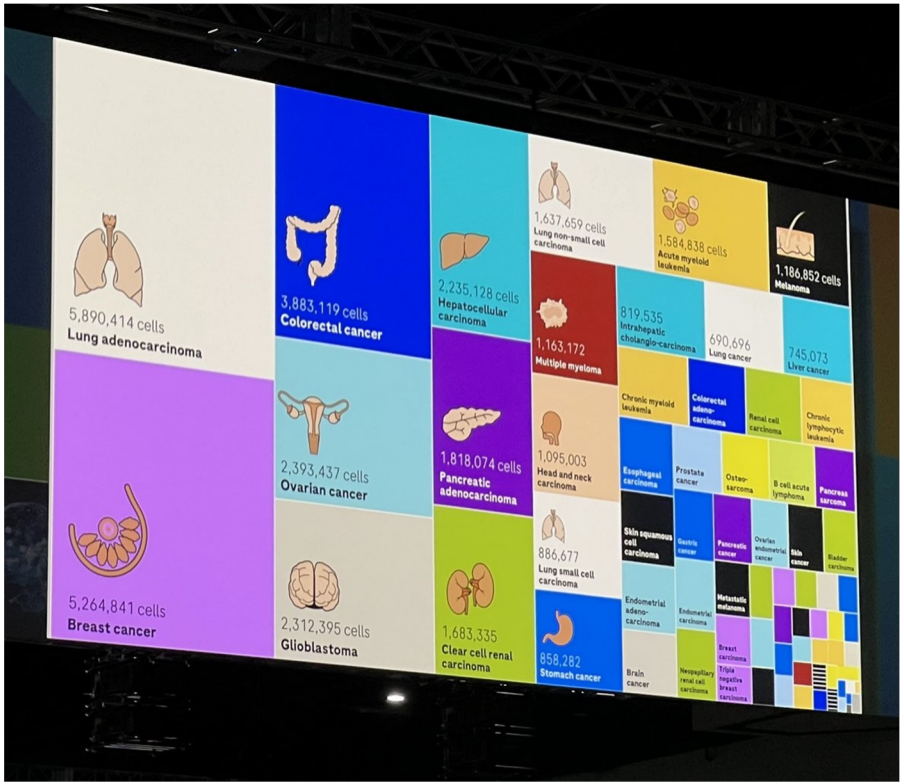
CRI Bioinformatics Bootcamp

R Workshop

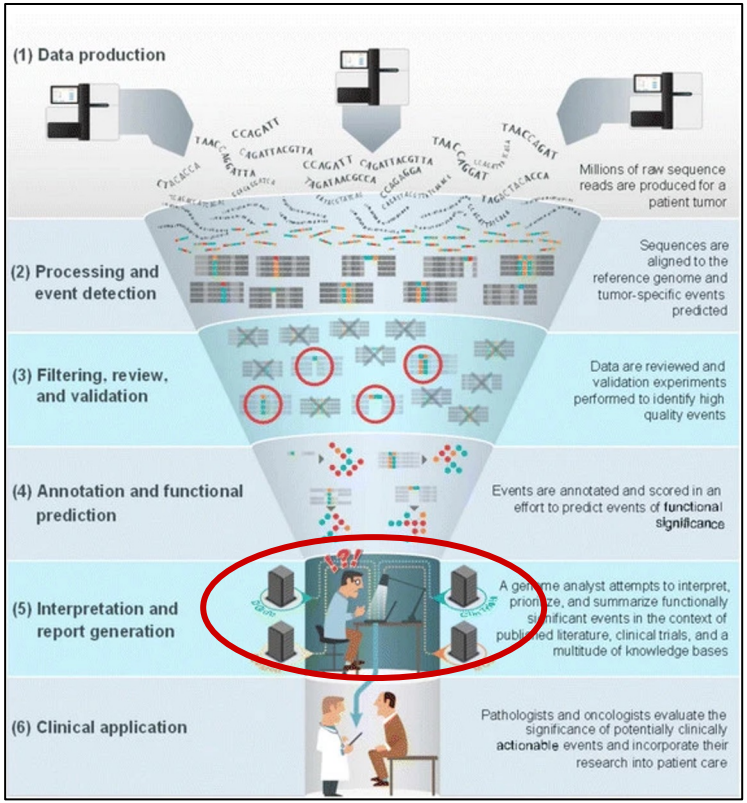
Why should we learn to code?



- Scale and accessibility of high-dimensional technologies
- Value in understanding how to generate and explore datasets
- Hypothesis-driven research through data generation

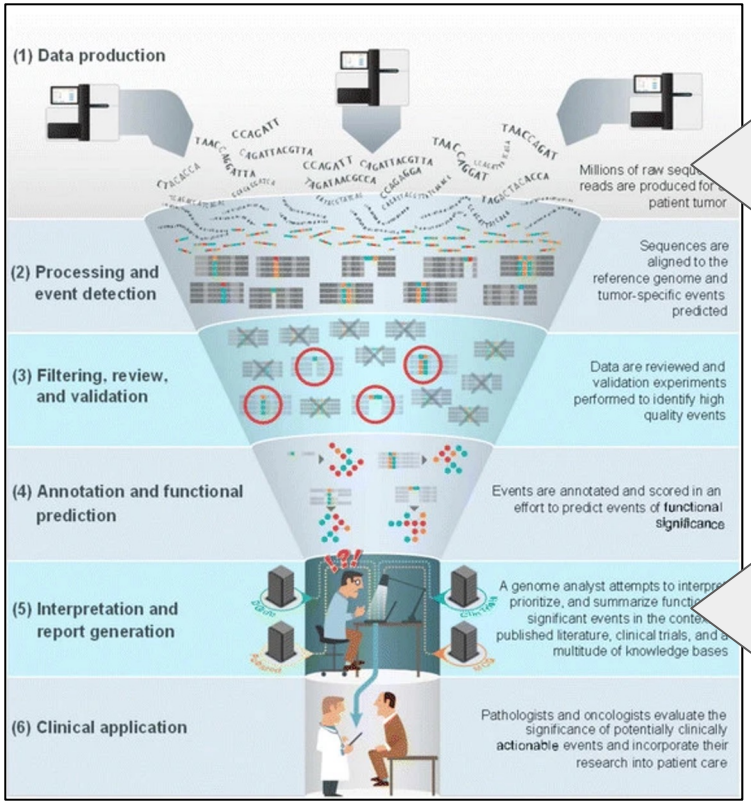
Aviv Regev, AACR 2024

How can we make sense of the data?



Good, et al. *Genome Biol* 2014

What is the question?



What kind of data is or should be generated?

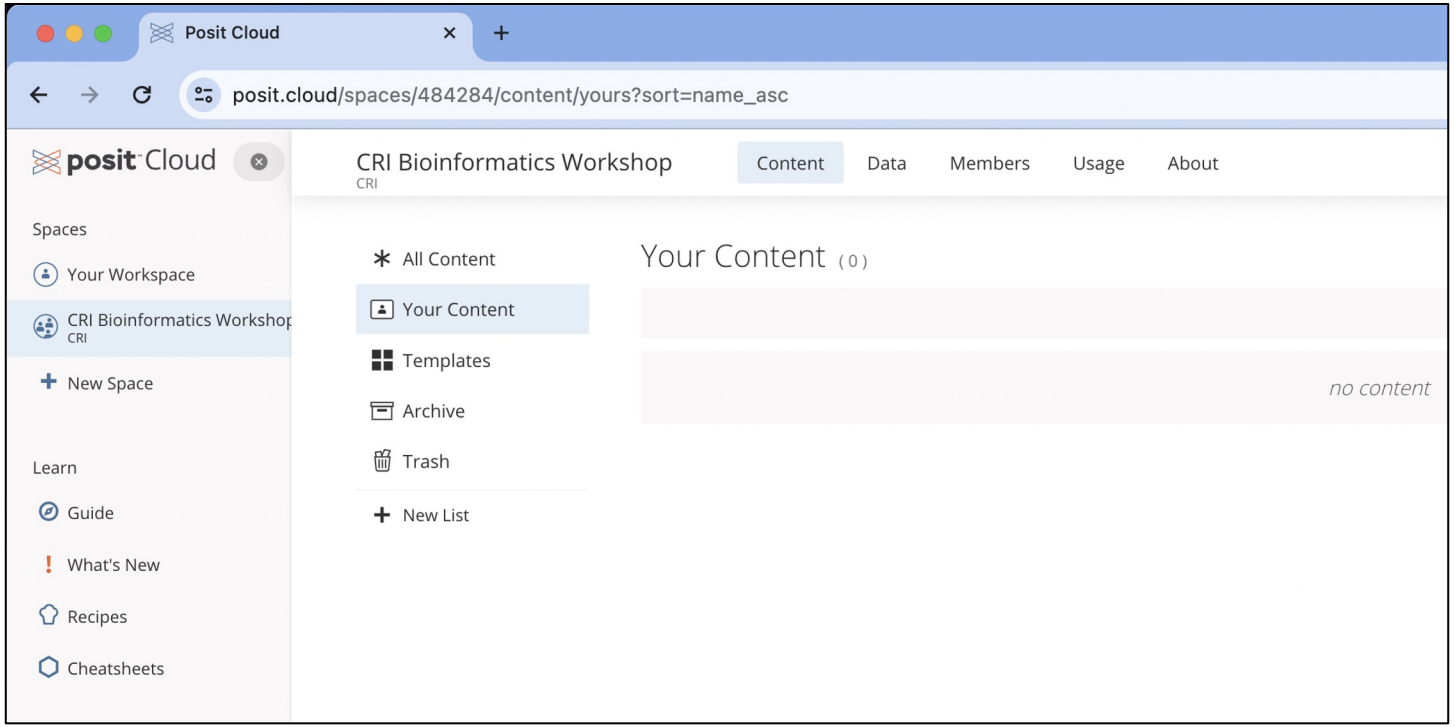
How do we alleviate the bottleneck?

What is the hypothesis?

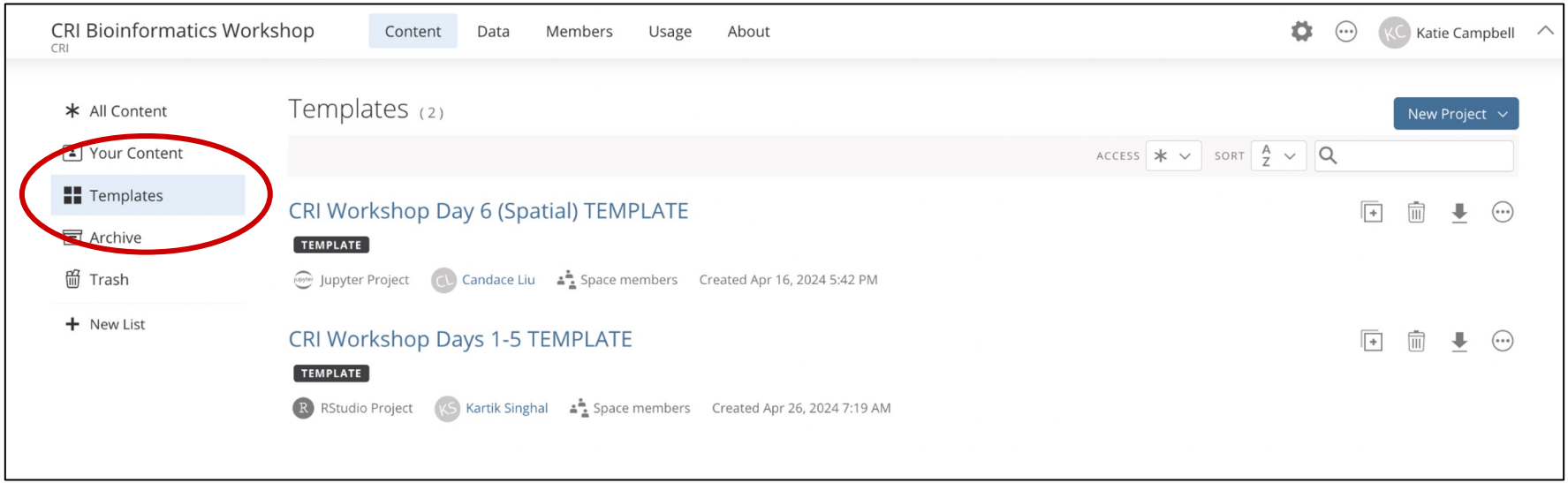
Goals for the R Workshop (Days 1-2)

- Learn enough to be a little dangerous
- Feel comfortable reading and running commands
- Understand what resources are available for when you leave

Using RStudio through Posit Cloud

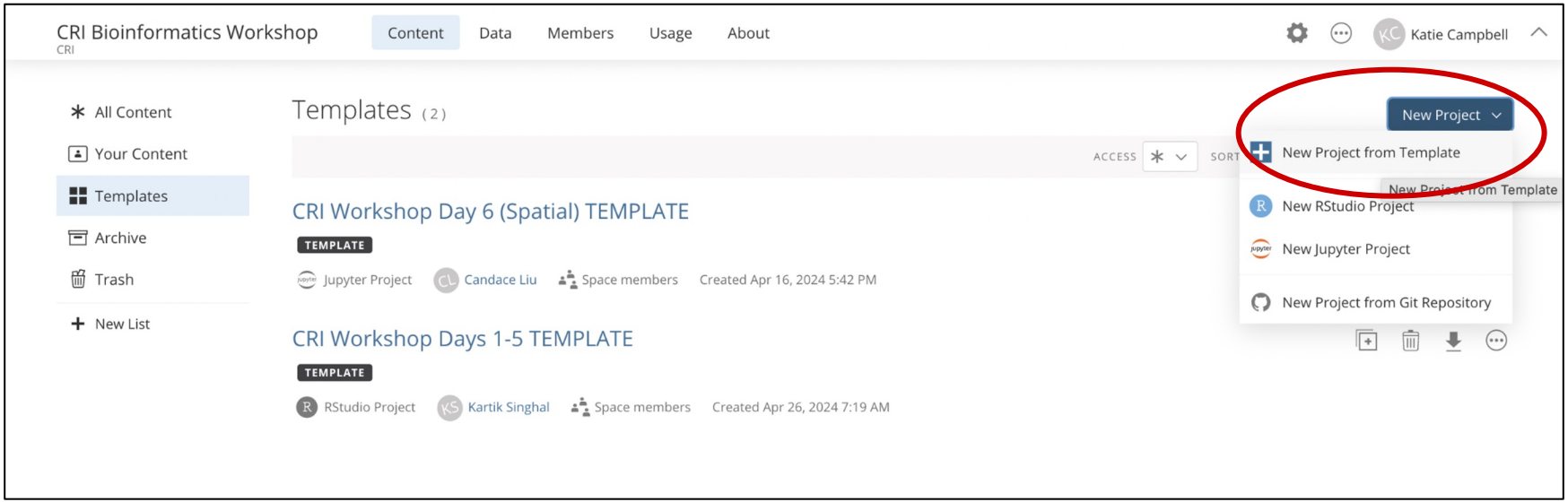


Getting started: Initiating your R project for this course



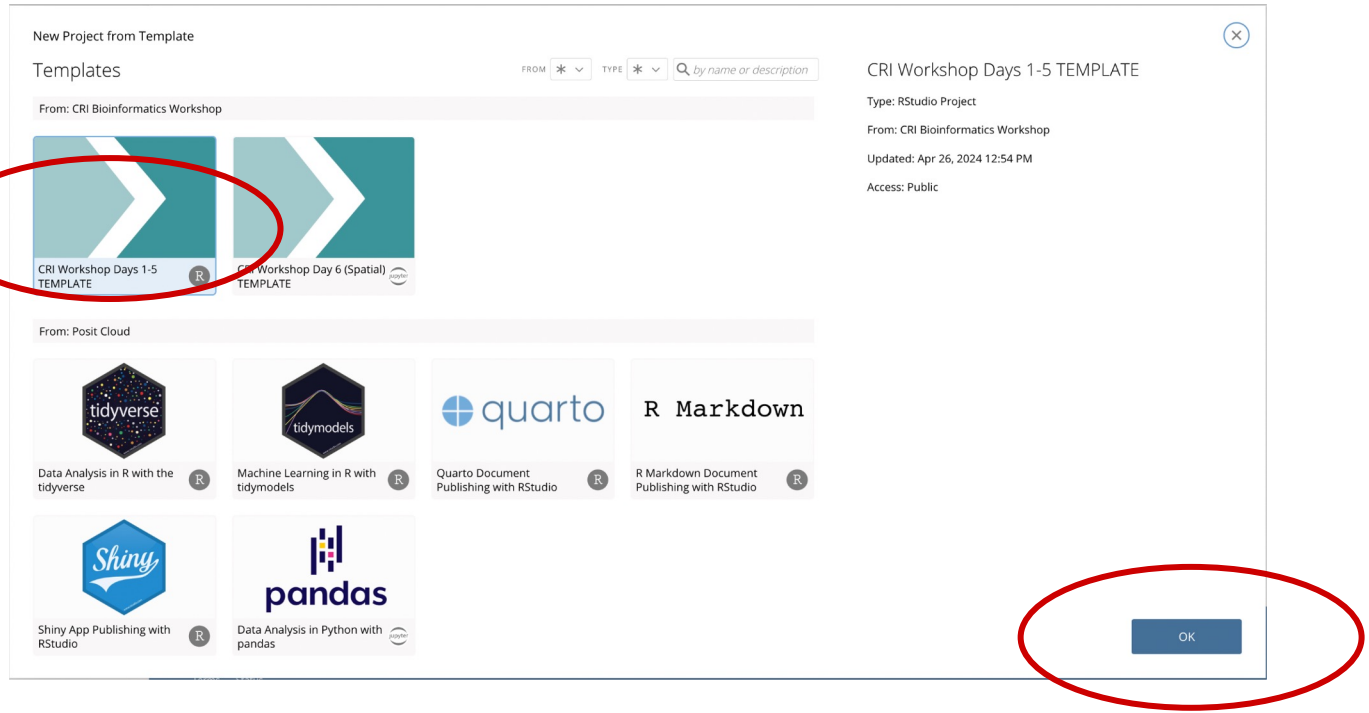
Step 1. Navigate to the **Templates** page

Getting started: Initiating your R project for this course



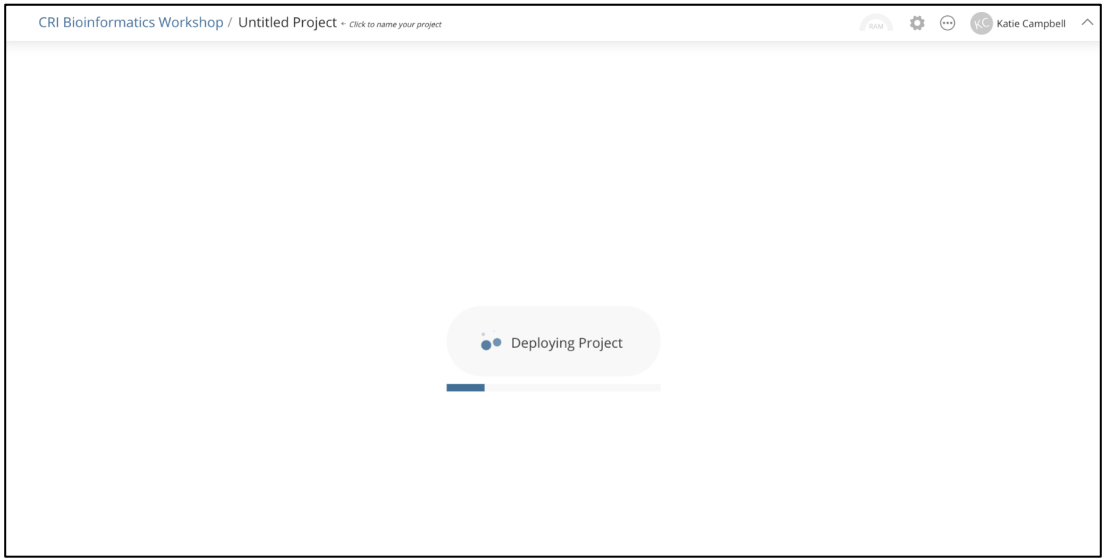
Step 2. Select **New Project > New Project from Template**

Getting started: Initiating your R project for this course



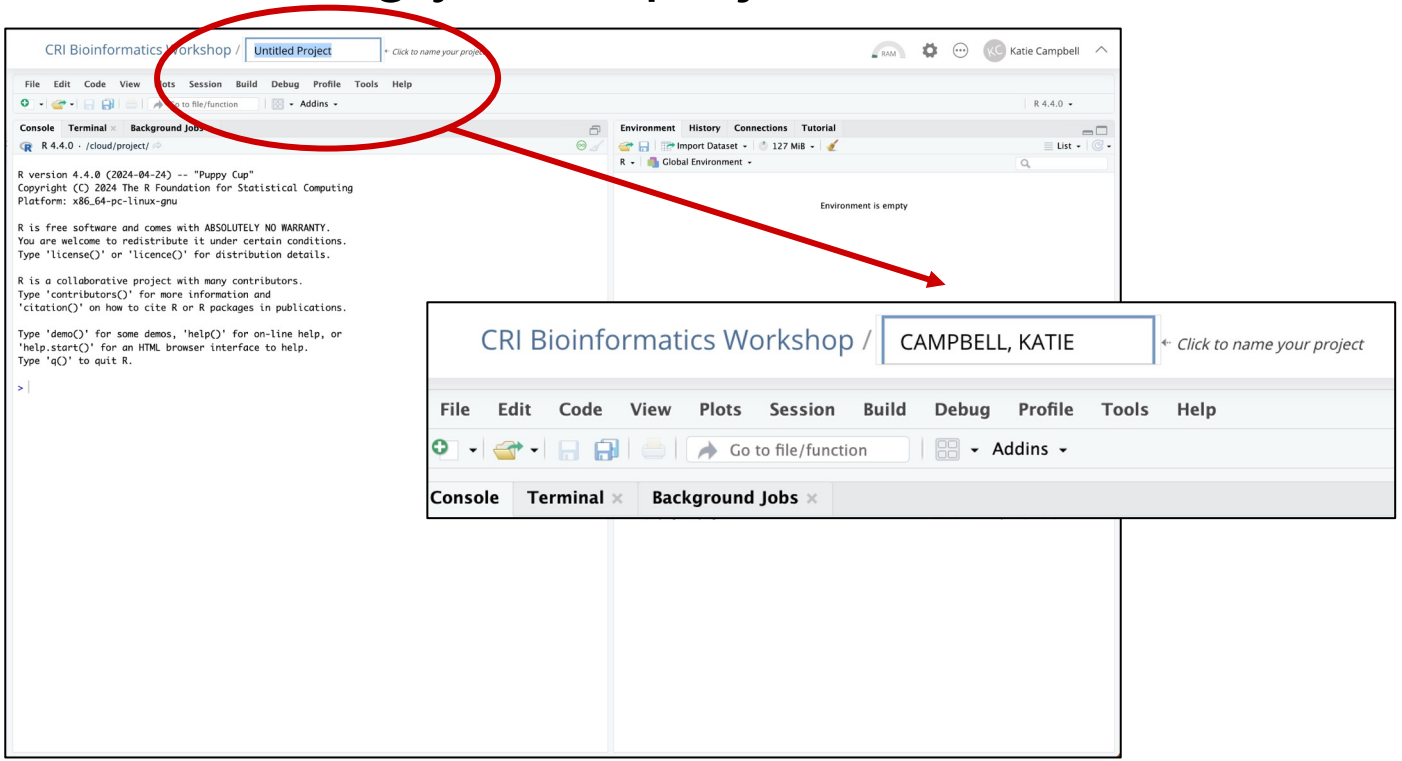
Step 3. Select **CRI Workshop Days 1-5 TEMPLATE > OK**

Getting started: Initiating your R project for this course



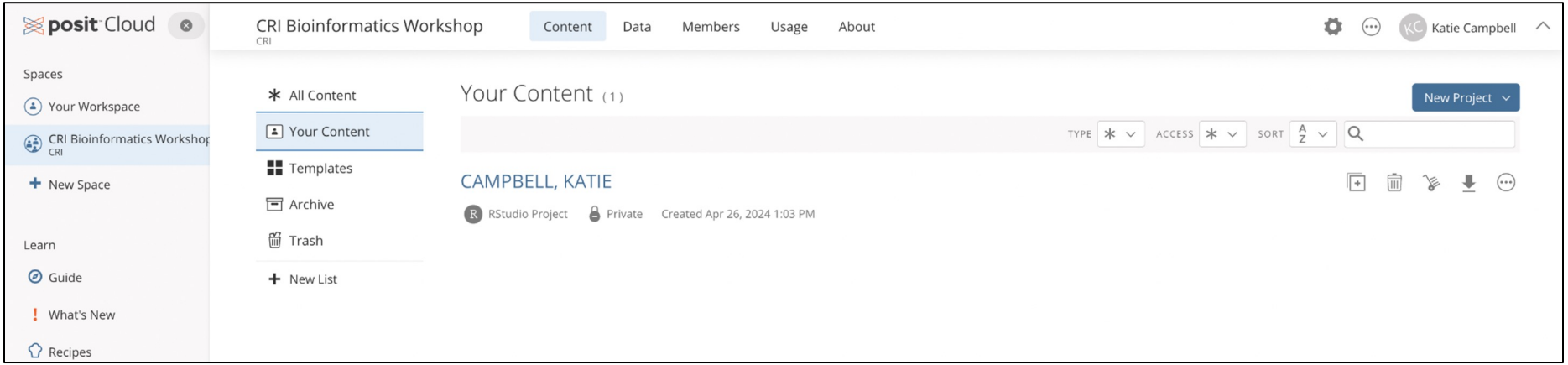
Step 4. Wait for the deployment of your project (currently called **Untitled Project**)

Getting started: Initiating your R project for this course



Step 5. Rename your project to your **LAST NAME, FIRST NAME**

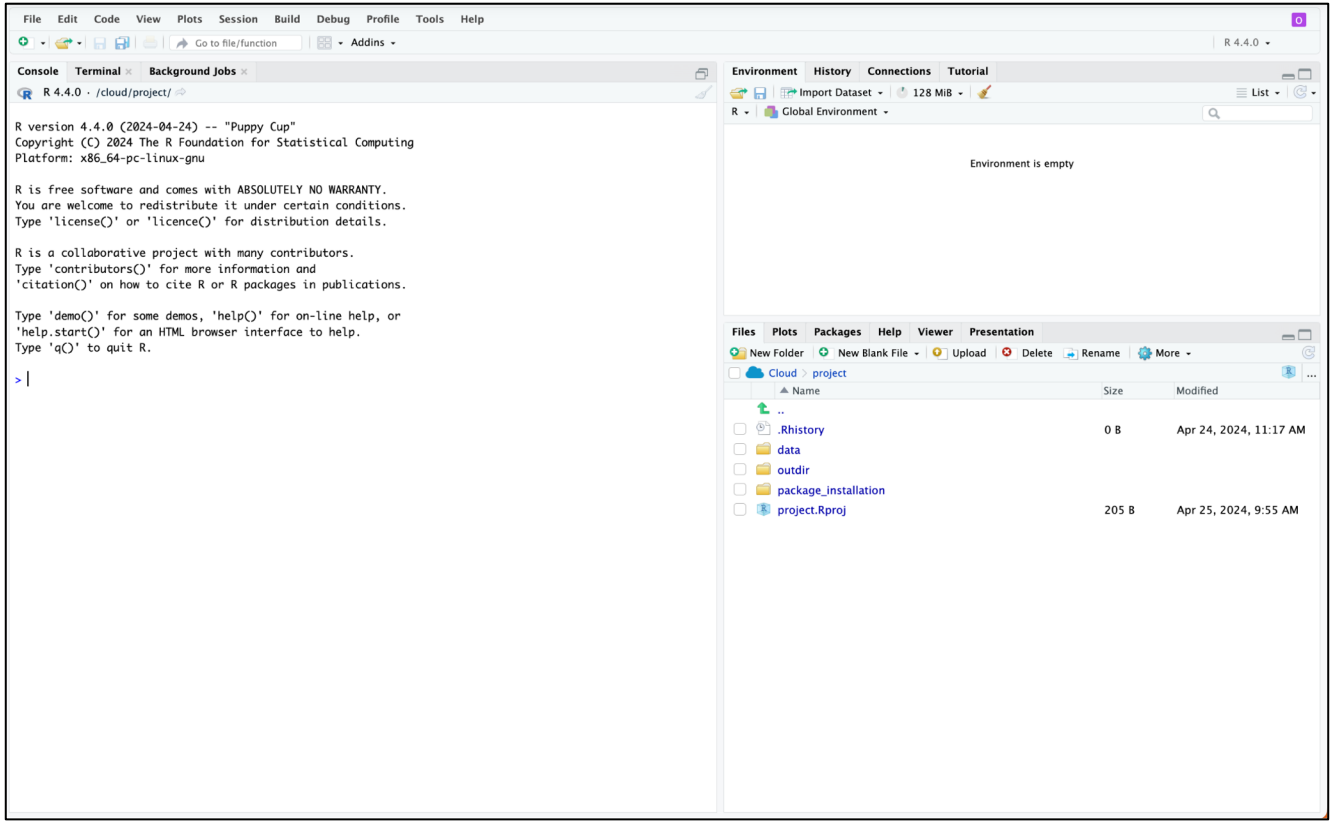
Only use ***your*** project for each day of this course



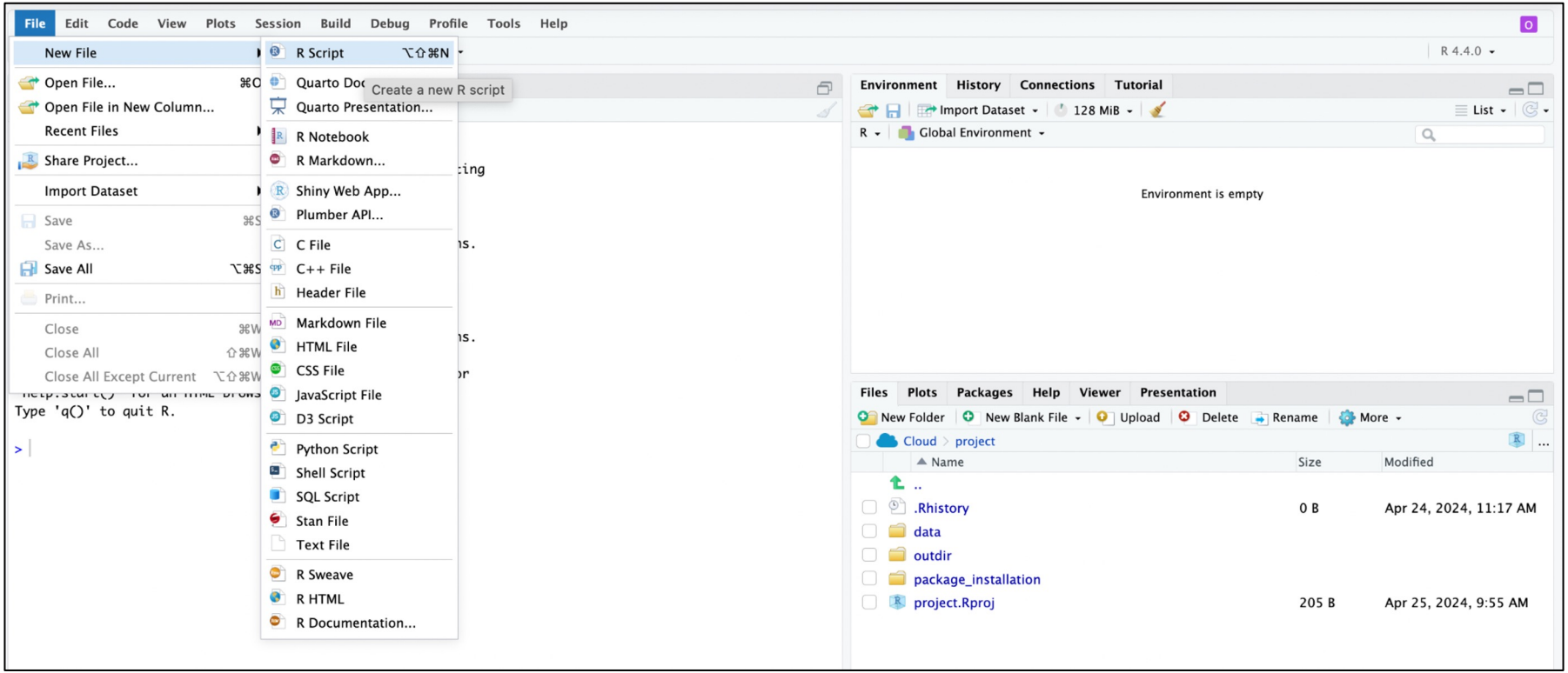
This project will be yours to save your work over the course of the next 5 days. Note that this is a private project for your access, but all TAs and instructors are also able to access your project.

Introduction to R and RStudio

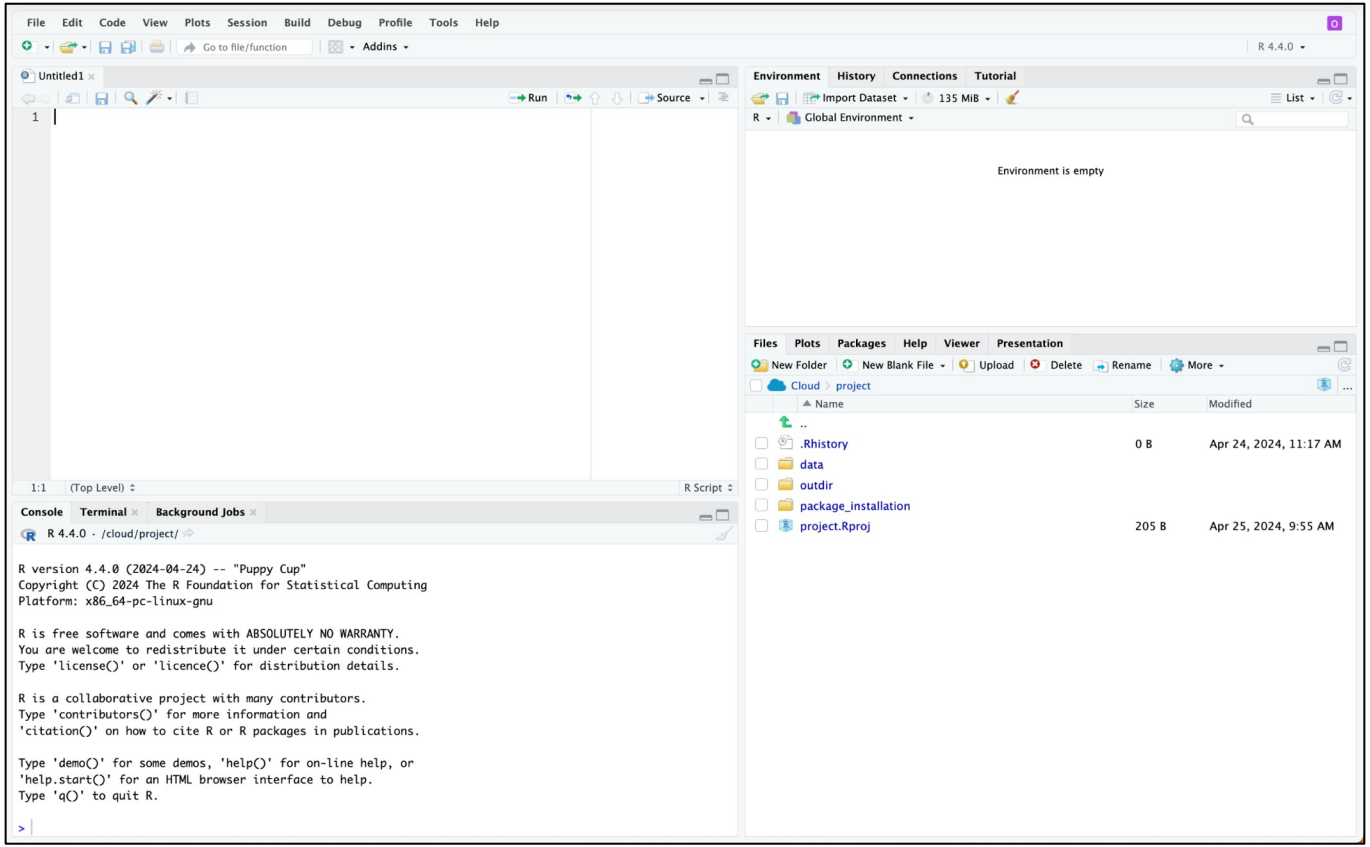
Orienting yourself with the RStudio interface



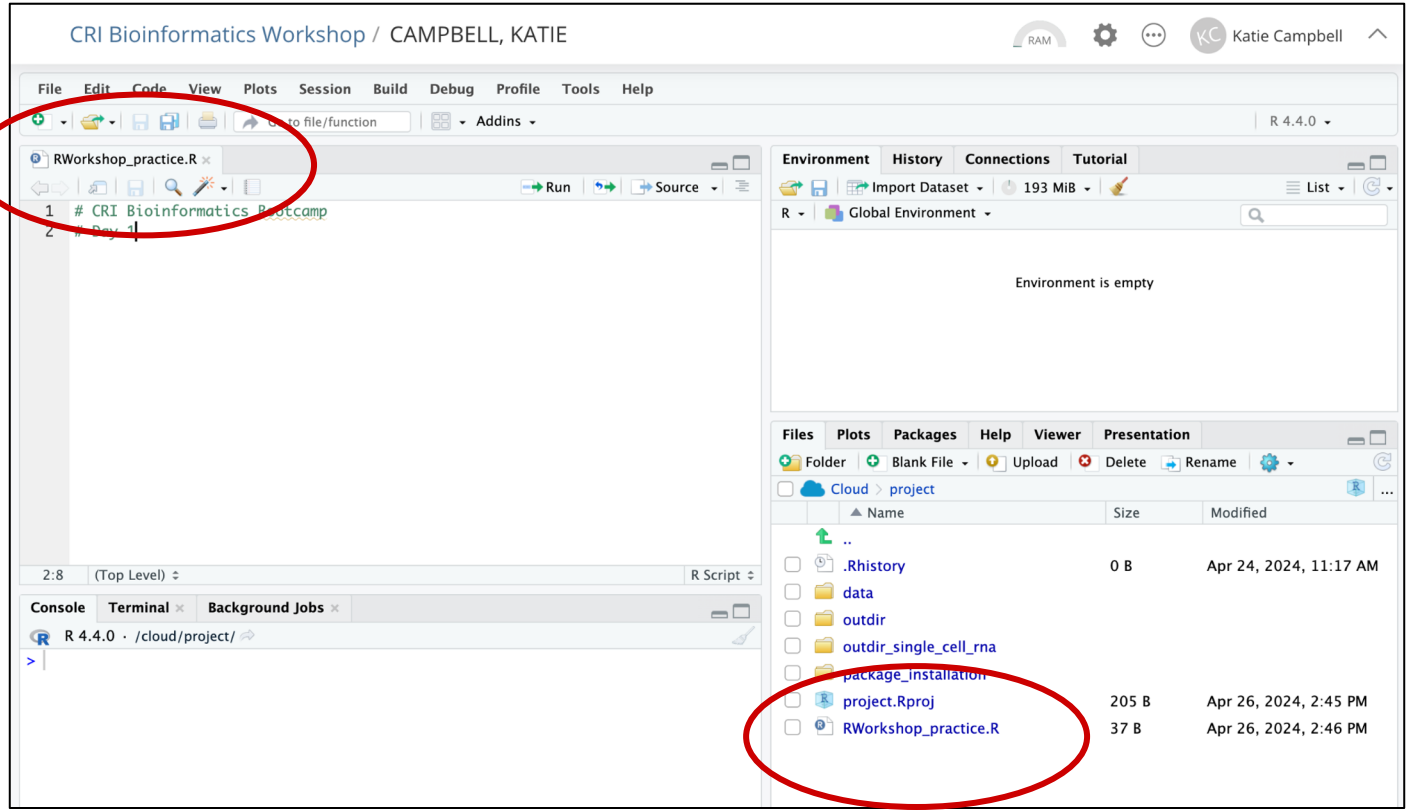
Creating a new R script



(Re)orienting yourself with the RStudio interface



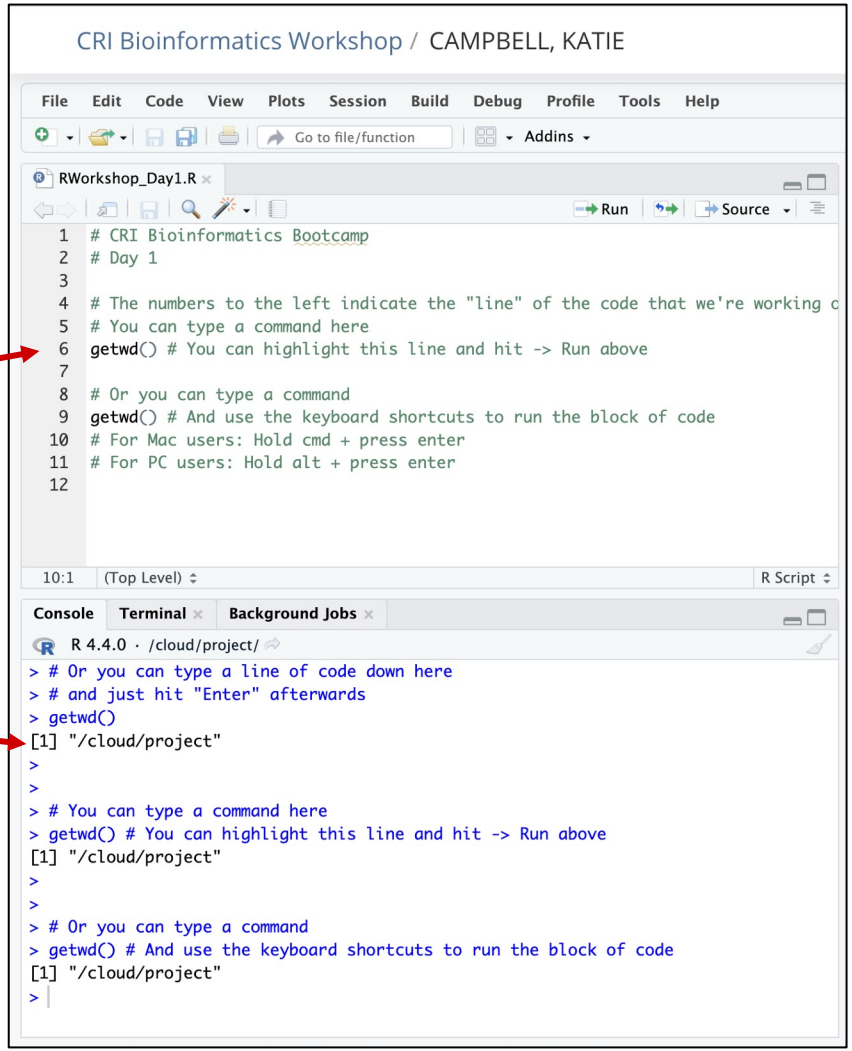
Saving your first Rscript in your project



Running your code

You can type commands directly into your script to save

Or type commands into the Console



The working directory: Orienting the filesystem of your computer

```r
# Get the current working directory
current_dir <- getwd()
print(paste("Current directory:", current_dir))

# List files in the current directory
files <- list.files()
print("Files in the current directory:")
print(files)

# Change the current directory
new_dir <- "path/to/new/directory"
setwd(new_dir)
print(paste("Changed directory to:", new_dir))
```

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```

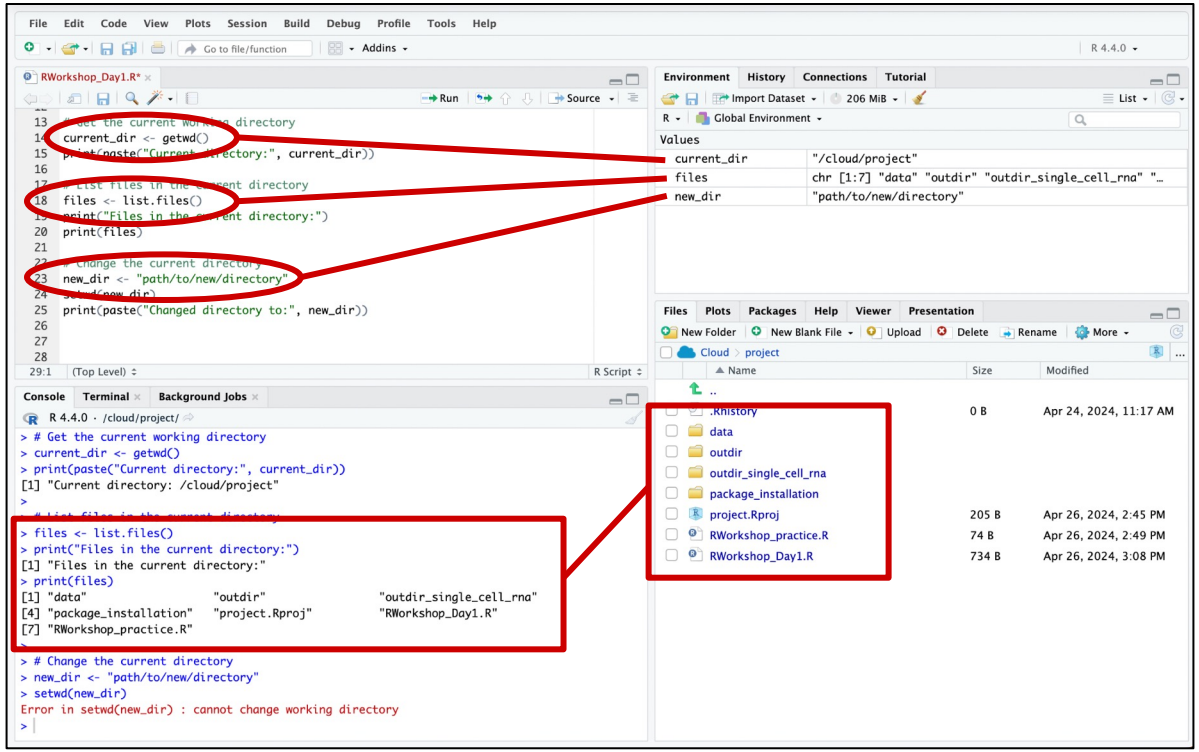
Functions are indicated by function_name(parameters)

paste() can be used to concatenate strings together

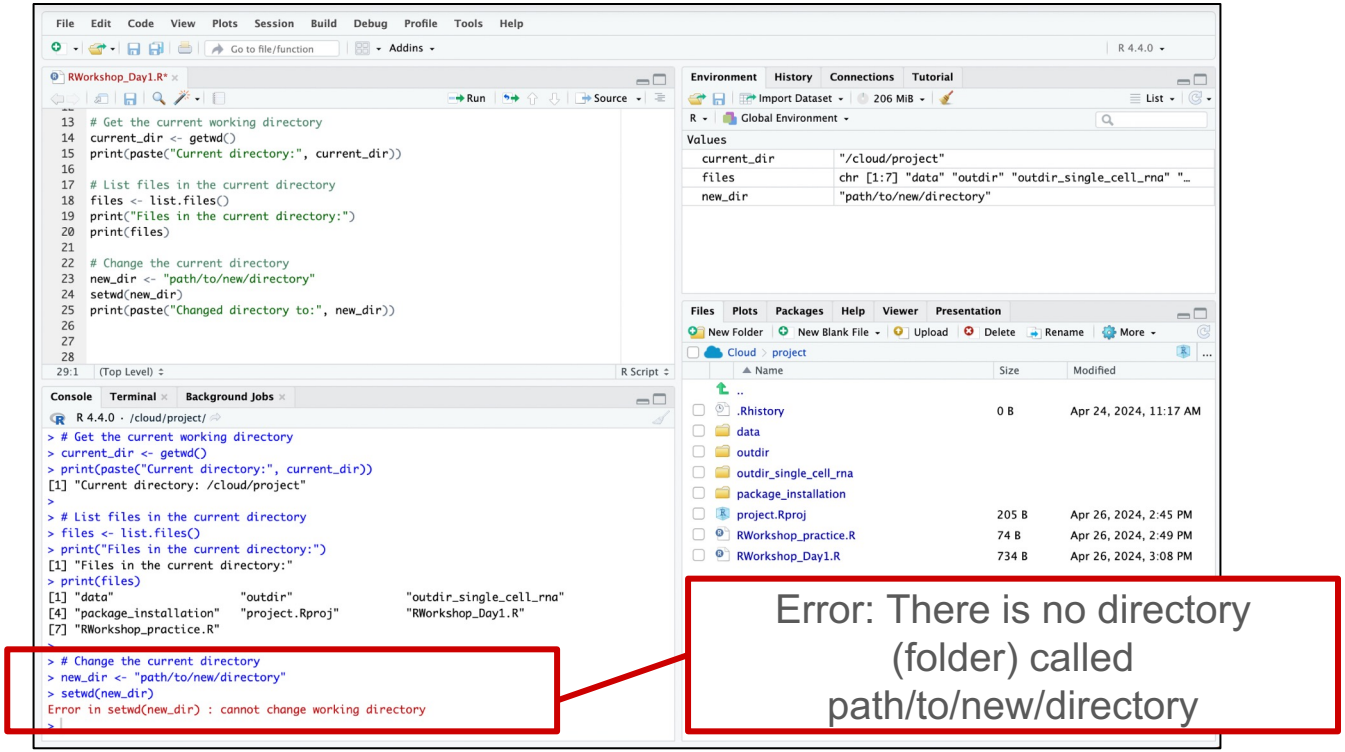
print() will print out what's inside the parentheses into the console

<- assigns the content on the right (the string) to the variable name on the left

Assignment of variables saves them within your environment



Be prepared for Errors…



Primitive Variables in R

- **Most basic data types**
  - Cannot be decomposed
- **Numeric (*Float*)**
  - Default variable for numbers
  - Includes floating point and integer
- **Integer**
- **Logical (*Boolean*)**
  - TRUE or FALSE
  - Conditional Statements
    - And/Or/Not
- **Character (String)**
  - Text data
  - “Hello world!”
- **Other types**
  - Raw (Byte Representation)
  - Complex
- **Data structures**
  - Composite of primitive

Numeric

```
# Numeric variable
num_var <- 10
print(num_var)  # Output: 10

# Arithmetic operations
result <- num_var * 2
print(result)  # Output: 20
```

Integer

```
# Integer variable
int_var <- 20L  # The 'L' suffix indicates an integer
print(int_var)  # Output: 20

# Integer arithmetic
result <- int_var / 5
print(result)  # Output: 4
```

Character

```
# Character variable
char_var <- "Hello, World!"
print(char_var)  # Output: Hello, World!

# Concatenation
new_string <- paste(char_var, "This is R programming.")
print(new_string)  # Output: Hello, World! This is R programming.
```

Vector and List

- **Vector**
  - 1 dimensional
  - Single data type
    - Numeric, Character, Logical
  - Efficient (pre-allocation)
  - Building Matrices
- **List**
  - Heterogeneous data
  - Dynamic
  - Creating Dataframes
- **Factor**
  - Categorical data
  - Levels
  - Memory efficient
    - Fixed set of values can be mapped with an integer

Vector

```r
# Creating a numeric vector
numeric_vector <- c(1, 2, 5.3, 6, -2, 4)

# Creating a character vector
character_vector <- c("apple", "banana", "cherry")

# Creating a logical vector
logical_vector <- c(TRUE, FALSE, TRUE, FALSE)

#Pre-allocate
my_vector <- numeric(10)
```

List

```r
# Creating a list with mixed types
my_list <- list(
  barcode = "AAAGCAAGTTACGCGC",
  umis = 25,
  seq = c("CASSLVLPGYTEAFF", "CASVGQGEYEQYF"),
  cell = TRUE,
)

# Accessing elements
print(my_list$barcode)       # Outputs "AAAGCAAGTTACGCGC"
print(my_list$seq)     # Outputs c("CASSLVLPGYTEAFF", "CASVGQGEYEQYF")
```

Matrix

- 2 dimensional
- Single data type
- *matrix()*
  - data
  - nrow
  - ncol
  - byrow
    - Whether to fill by rows or columns
  - dimnames'
- Access elements by row and column
- Matrix operations

Creating a Matrix:

```
# Create a matrix with random values
mat <- matrix(rnorm(20), nrow = 4, ncol = 5)
```

Matrix operations

```
element <- mat[1, 2]

# Calculate row sums
row_sums <- rowSums(mat)

# Calculate column sums
col_sums <- colSums(mat)

# Create another matrix
mat2 <- matrix(rnorm(20), nrow = 5, ncol = 4)

# Perform matrix multiplication
mat_mult <- mat %*% mat2

# Transpose the matrix
mat_transpose <- t(mat)

# Select the first two rows
first_two_rows <- mat[1:2, ]

# Select the first three columns
first_three_cols <- mat[, 1:3]
```

Dataframe

- **What is a dataframe?**
  - “Excel”-like structure
  - Heterogeneous data
  - Labeled columns and rows
  - *data.frame()*
- **Using the dataframe**
  - Accessing columns by name
    - df$column_name
  - Access by row and column
    - df[i,j]
  - Filtering
    - subset(df, column_name > 25)
  - Add columns
    - df$col2 <- c(1,2,3)
  - Add rows
    - df <- rbind(df, c(“w”,2,True))
  - Modify values
    - df[i,j] <- 25

```r
# Create a data.frame
df <- data.frame(
  barcode = c("AAACCTGAGAAGGCCT-1", "AAACCTGCAAGTTGTC-1", "AAACCTGCACCGATAT-1"),
  phenotype = c("TEMRA", "Naive", "Dysfunctional"),
  treatment = c("Pre", "Post", "Pre"),
  response = c("R","NR","R")
)

result <- df$phenotype)
print(result) # prints "TEMRA","Naive","Dysfunctional"

result <- df[1,2]
print(result) # prints "TEMRA"

result <- subset(df,treatment=="Post")
print(result) # prints AACCTGCAAGTTGTC-1 Naive Post NR

df$site <- c("Tumor","Tumor","Blood")

df <- rbind(df,c("AAACCTGCACCGATAT-1","Naive","Post","R","Tumoxr"))

df[4,5] <- "Tumor"
```

```
> df
             barcode     phenotype treatment response  site
1 AAACCTGAGAAGGCCT-1         TEMRA       Pre        R Tumor
2 AAACCTGCAAGTTGTC-1         Naive      Post       NR Tumor
3 AAACCTGCACCGATAT-1 Dysfunctional       Pre        R Blood
4 AAACCTGCACCGATAT-1         Naive      Post        R Tumor
```

String Manipulation

- Strings vs substrings
  - Substring is a sequence of text within a string.
  - “Hello” is a substring of “Hello world!”
- Case conversion
  - Uppercase and lower matter, R is case-sensitive
- Find and replace
  - Find a substring and replace it with another substring
  - Examples: ID matching, motifs, etc.
- Length calculation
  - Sequence length
- Stringr package (tidyverse)
  - str_length
  - str_to_upper
  - str_locate
  - str_sub
  - str_replace and str_replace_all
- Base: grep, grepl, gsub
- Regular expressions
  - Search pattern
  - “hell*olrd!” matches “hello world!”

Length Calculation

Determining the length of sequences.

```
sequence_length <- str_length(sequence)
print(sequence_length)
```

Case Conversion

Converting uppercase to lowercase, or vice versa.

```
sequence_upper <- str_to_upper(sequence)
print(sequence_upper)
```

Substring Extraction

Extracting parts of sequences, such as cutting out genes or regions of interest.

```
extracted_sequence <- str_sub(sequence, 3, 8)
print(extracted_sequence)
```

Replacing Substrings

Modifying sequences by replacing specific nucleotides or amino acids.

```
dna_sequence <- "ATGCGTACGTTGACT"
rna_sequence <- str_replace_all(dna_sequence, "T", "U")
print(rna_sequence)
```

Logical operations

- Evaluate to True or False
  - logical data type
- Use case
  - Conditional statements
    - if / else
  - Filtering data
    - subset(df,treatment=”post”)
- Comparisons
  - Equal: ==
  - Not equal: !=
  - Greater Than: “>”
  - Less Than “<”
  - %in%
    - Test inclusion of one vector in another
- Boolean statements
  - and: both are true “&”
  - or: atleast one is true “|”
  - not: evaluates to false “!”

```r
# Greater than
result_gt <- x > y
print(result_gt)  # Output: FALSE

# Less than
result_lt <- x < y
print(result_lt)  # Output: TRUE

# Equal to
result_eq <- x == y
print(result_eq)  # Output: FALSE

# Not equal to
result_neq <- x != y
print(result_neq)  # Output: TRUE
```

```r
# Logical operations
a <- TRUE
b <- FALSE

# AND operation
result_and <- a & b
print(result_and)  # Output: FALSE

# OR operation
result_or <- a | b
print(result_or)   # Output: TRUE

# NOT operation
result_not <- !a
print(result_not)  # Output: FALSE
```

```r
x <- 3
if (x > 5) {
  print("x is greater than 5")
} else if (x == 5) {
  print("x is equal to 5")
} else {
  print("x is less than 5")
}
```

Writing your own functions

- Encapsulate reusable block of code for a specific task
- Elements of a function
  - Name
  - Input (Arguments)
  - Body
  - Output (Optional)
    - Writing to a file
    - Plotting
- Why write functions?
  - Breaking down larger problems
  - Reusability
  - Minimizing errors
  - Maintainability

```
function_name <- function(argument1, argument2, ...) {
  # Function body
  # Perform operations
  # Return a value (optional)
}
```

```
# Define a function to calculate the square of a number
square <- function(x) {
  return(x^2)
}

# Call the function
result <- square(5)
print(result)  # Output: 25
```

Manipulating data frames in tidyverse

What “Tidy” means:

- Each variable is a column
- Each observation is a row
- A table is a consistent set of observations for a known set of variables.

Tidyverse Packages for Data Manipulation:

- **dplyr**: Provides a grammar of data manipulation for data frames, enabling easy filtering, selecting, mutating, summarizing, and arranging of data.
- **tidyr**: Offers tools for reshaping and tidying messy datasets, such as `gather()` and `spread()` functions for converting between wide and long formats.
- **ggplot2**: Allows for the creation of sophisticated data visualizations using a layered grammar of graphics.

```r
library(tidyverse)

# Create a tibble (tidyverse version of data.frame)
df <- tibble(
  barcode = c("AAACCTGAGAAGGCCT-1", "AAACCTGCAAGTTGTC-1", "AAACCTGCACCGATAT-1"),
  phenotype = c("TEMRA", "Naive", "Dysfunctional"),
  treatment = c("Pre", "Post", "Pre"),
  response = c("R", "NR", "R")
)
```

Nice features:

- Printing
- Variable information
- Extracting a column for a tibble yields another tibble
  - data.frame will yield a vector
- Row index not added by default
  - Explicit id column

Tidyverse Operations

Filter

- Conditional subsetting
- Multi-condition

Mutate

- Add new columns
- Modify columns
- vectorized
    - Applied to column

Pipe: “%>%”

- Chaining commands
- Readability

```r
# Filter rows where treatment is "Post"
result <- df %>%
  filter(treatment == "Post")
print(result) # prints the row with treatment "Post"

# Add a new column 'site' and update its values
df <- df %>%
  mutate(site = c("Tumor", "Tumor", "Blood"))

# Add a row to the dataframe
df <- df %>%
  add_row(
    barcode = "AAACCTGCACCGATAT-1",
    phenotype = "Naive",
    treatment = "Post",
    response = "R",
    site = "Tumor"
  )
```

Reading in your own files

CSV/TSV

- read.csv or read_csv (“tidyverse”)
- Comma separated or tab separated

Excel

- readxl()
- sheet and cell selection

Understanding the structure of your data

- str()
  - Shows human readable structure of any R object (data.frame, tibble, etc.)
- summary()
  - Adaptive to type of object
  - Summarizes mean, median, etc. for numeric data
  - Shows model diagnostics

```r
# Read a CSV file into a data frame
data <- read_csv("path/to/your/file.csv")

# View the structure of the data frame
print("Structure of the data frame:")
print(str(data))

# View the first few rows of the data frame
print("First few rows of the data frame:")
print(head(data))
```

```r
# Read a specific sheet by name
data_sheet2 <- read_excel("path/to/your/file.xlsx", sheet = "SecondSheet")

# Read a specific sheet by index
data_sheet3 <- read_excel("path/to/your/file.xlsx", sheet = 3)

# Read specific cells, e.g., A1:C10 from the first sheet
data_range <- read_excel("path/to/your/file.xlsx", range = "Sheet1!A1:C10")
```

```r
# Create a numeric vector
numeric_vector <- c(23, 45, 67, 23, 89, 45, 12, 54, NA, 78)

# Use summary to get a statistical overview of the vector
summary_result <- summary(numeric_vector)
print(summary_result)
```

```
> print(summary_result)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.    NA's
  12.00   23.00   45.00   48.44   67.00   89.00       1
```

Hands-on: Reading, writing, and interpreting data structures

- These slides are available on the course website under **Resources: During the course: Lecture slides**
  - Set up your project through Posit
- Start coding
  - Start your first Rscript
  - Work through the blocks of code under the **Course: Introduction to R** page
    - Review the details on how the code works in the Lecture slides for assistance
    - Put a post-it on your laptop if you get stuck, indicating for a TA to come up to you
    - Work through the blocks of code on this page, practicing in both your Rscript and the terminal
  - Taking the next step
    - There are a list of **Additional exercises** at the bottom of the page for you to try on your own

Goal: Start building your R vocabulary