Introduction to R

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R is a high-level programming language and open-source software environment extensively utilized in bioinformatics for a wide array of applications. Its popularity in this field stems from its robust data manipulation capabilities and an extensive library of statistical and bioinformatics packages. Researchers in bioinformatics use R to analyze biological data, perform genomic sequencing, identify gene expressions, and conduct statistical analyses on large datasets. Moreover, R's powerful visualization tools enable the creation of compelling graphs and charts to visualize genomic patterns and analyze biological networks. As a result, R plays a vital role in bioinformatics, empowering scientists to unravel complex biological insights and facilitate advancements in genomics, proteomics, and other critical areas of life sciences.

In this section we are going to dive deeper into R. We will cover various topic to help you get familiar with its core concepts:

- 1) Data Types in R
- 2) Objects in R
- 3) Data Management
- 4) Basic Statistical Functions
- 5) Visualizations
- 6) Conditional Statements, Loops, and Functions in R
- 7) Working with Packages & Libraries within R

Data Types in R

Like Python, R allows you to store and create variables. Thus, variables can be of different types. The syntax for declaring a variable is show below:

```
x <- 3 # arrow sign
y = 7 # equals sign
```

Notice how in your environment on RStudio (top right) you will see key value pairs of the variable name and its corresponding values. This is extremely useful as it allows you to consistently keep track of your variables and can help to debug.

There are a few basic data types in R: numeric, character, logical, integer, complex:

-numeric: these type of variables can represent integers and decimal numbers - character: Represents text or strings of characters. Strings are enclosed in quotes, either single (') or double ("). - logical: Represents Boolean values, which can be either TRUE or FALSE - Integer: Represents whole numbers. Integers are a subset of numeric values but have a distinct data type in R. - complex: Represents complex numbers with both real and imaginary parts. Complex numbers are written in the form a + bi, where a represents the real part and b represents the imaginary part.

Run the following snippet to get a better understanding of these types of data types. Note that the class() function gives you more details on the data type of a variable if you ever need to know more. (We Will cover more about functions later on)

```
x <- 13
class(x) # this will output numeric but it is also an integer!
[1] "numeric"
x < -45.3
class(x)
[1] "numeric"
x <- TRUE
class(x)
[1] "logical"
x <- "Hello World"
class(x)
[1] "character"
x <- 98i
class(x)
[1] "complex"
```

Quick did you notice that the value of x in your environment changed several times? This is important because you can very easily override a variable in R, so be careful!!

Arithmetic

These built-in data types provide the core methods of computation. R allows various forms of arithmetic such as addition, division, modulo, etc.

```
x <- 10
y <- 3
x + y
[1] 13
х - у
[1] 7
x * y
```

[1] 30

```
x / y

[1] 3.333333

x^y

[1] 1000

y %% x
```

[1] 3

Hopefully this was relatively simple to follow as the syntax is actually quite similar to Python!

Relational Operators

The similarity between Python and R syntax does not stop there though. The syntax for relational operation is also the same! Relational operators define some kind of a relation between variable like greater than, equal to, etc.

```
x < -50

y < -35

x == y
```

[1] FALSE

```
x != y
```

[1] TRUE

```
x < y
```

[1] FALSE

```
x <= y
```

[1] FALSE

```
y > x
```

[1] FALSE

```
y >= x
```

[1] FALSE

Notice how the outputs of these operators are either TRUE or FALSE. This will come in handy when we talk about control flow in R later on in this module, so keep this in mind!

Objects in R

In R, an object is a fundamental concept that represents data or information stored in memory. It serves as a storage box for holding values (like numbers, text, vectors, matrices, lists, and more complex data structures). Each object in R has a unique name assigned to it, allowing for easy reference and manipulation. Objects in R are not just simple variables but are entities with specific attributes and behaviors. They can be created, modified, and interacted with during the execution of an R program. Objects in R enable users to store, organize, and process data efficiently, supporting a wide range of operations and analyses.

So technically simple data types like integers are indeed objects. But bjects can get a lot more complicated. Lets learn of a few:

Vectors

In R, vectors are one-dimensional objects that can store multiple values of the same data type. They are a fundamental data structure and can be created using the c() function or generated by other functions. Here's an explanation along with some code examples:

```
numbers <- c(1, 2, 3, 4, 5)
```

Here we create a vector called numbers, notice how it is also stored in your R environment.

There are other ways to initialize vectors as well:

```
years <- seq(2000, 2023)
years</pre>
```

```
[1] 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 [16] 2015 2016 2017 2018 2019 2020 2021 2022 2023
```

```
(x <- rep(FALSE, 10)) # wrapping in an extra set of parentheses will
```

[1] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE

```
# automatically print out the result
(y <- 1:10)</pre>
```

```
[1] 1 2 3 4 5 6 7 8 9 10
```

Since vectors are a sequence of numbers you and easily access elements using bracket notation [].

```
years[4]
```

[1] 2003

```
years[c(5, 7, 9)]
```

[1] 2004 2006 2008

```
years[5:9]
```

[1] 2004 2005 2006 2007 2008

```
years[seq(3, 5)]
```

```
[1] 2002 2003 2004
```

You can pass in specific index values to extract values from, you can also pass in another vector too! NOTE: Unlike Python R is not zero index, so the first element of a vector is at index position 1 (not 0)

```
years[1]
```

[1] 2000

Matrix

In R, a matrix is a two-dimensional data structure that represents a rectangular grid of elements. It consists of rows and columns, where each element holds a value of the same data type. Matrices are useful for organizing and manipulating tabular data, as well as for performing matrix algebra and mathematical operations.

Unlike vectors, which are one-dimensional, matrices provide a structured way to store data in a grid-like format. The rows of a matrix represent observations or cases, while the columns represent variables or attributes. Each element within the matrix can be accessed using its row and column index.

Matrices in R can contain numeric values, characters, logical values, or other data types. They are created using the matrix() function, specifying the data elements, the number of rows (nrow), and the number of columns (ncol). Once created, elements within a matrix can be accessed, modified, and manipulated using indexing operations.

The matrix function has the following parameters: (data = NA, nrow = 1, ncol = 1, byrow = FALSE, dimnames = NULL)

- data: a vector of element for the matrix
- norw and ncol: the row x col dimensions of the matrix
- dimnames: optional names for the rows and cols of your matrix
- byrow: when the matrix is filled with data, FALSE will have data populated by columns and TRUE will have the data populated by column

```
(y <- matrix(50:61, nrow=4, ncol=3))
```

```
[,1] [,2] [,3]
[1,]
        50
              54
                    58
[2,]
        51
              55
                    59
[3,]
        52
              56
                    60
[4,]
        53
                    61
```

```
(y <- matrix(50:61, nrow=4, ncol=3, byrow=TRUE))</pre>
```

```
[,1] [,2] [,3]
[1,]
       50
             51
[2,]
       53
             54
                   55
[3,]
       56
                   58
             57
[4,]
       59
             60
                   61
```

Below is how you can label the rows and columns

```
Col 1 Col 2 Col 3
         50
               51
                     52
Row 1
         53
               54
                      55
Row 2
Row 3
               57
                      58
         56
Row 4
         59
               60
                      61
```

```
# sometime when you have a lot of parameters it is best to split
# into different lines
```

In case there is ever a function that you do not remember the parameters for you can:

- 1) Type in the function name to the R console (bottom left)
- 2) Search it up in the "Help" tab, bottom right

You can also use the help() function:

```
help(matrix)
```

```
starting httpd help server ... done
```

Below we will go over how to index a matrix using the [row, col] syntax:

```
(y[1,]) # this will get the first row in the y matrix
```

```
Col 1 Col 2 Col 3
50 51 52
```

```
(y[3,]) # this gets the third row
```

```
Col 1 Col 2 Col 3
56 57 58
```

You can also use the labels of the rows and columns:

```
y["Row 1",]

Col 1 Col 2 Col 3
    50    51    52

y[,"Col 2"]

Row 1 Row 2 Row 3 Row 4
    51    54    57    60

y["Row 2", "Col 3"] # you can get a specific element in the matrix

[1] 55
```

Arthmetic and Relation with Vectors and Matricies

Now that we covered the basics of vectors and matrices lets apply similar concepts that we previously learned to them.

```
x <- c(1,2,3,4,5)
y <- c(6,7,8,9,10)
x + y

[1] 7 9 11 13 15
x - y

[1] -5 -5 -5 -5 -5
x * y

[1] 6 14 24 36 50
x / y
```

[1] 0.1666667 0.2857143 0.3750000 0.4444444 0.5000000

Notice how for vectors though the syntax doesn't change too much the operation is applied to each element of the vector element wise

```
(y <- matrix(13:24, nrow=4, ncol=3))</pre>
     [,1] [,2] [,3]
[1,]
       13
            17
                 21
[2,]
                 22
       14
            18
[3,]
       15
            19
                 23
[4,]
       16
            20
                 24
x + y
     [,1] [,2] [,3]
[1,]
       14
            22
                 30
[2,]
       16
            24
                 32
[3,]
       18
            26
                 34
[4,]
       20
            28
                 36
cat("\n")
     [,1] [,2] [,3]
[1,] -12 -12 -12
     -12 -12 -12
[2,]
[3,] -12 -12 -12
[4,] -12 -12 -12
cat("\n")
x * y
     [,1] [,2] [,3]
            85 189
[1,]
       13
[2,]
       28
                220
           108
[3,]
       45
           133
                253
[4,]
       64
           160
                288
cat("\n")
x / y
                      [,2]
           [,1]
                                 [,3]
[1,] 0.07692308 0.2941176 0.4285714
[2,] 0.14285714 0.3333333 0.4545455
[3,] 0.20000000 0.3684211 0.4782609
[4,] 0.25000000 0.4000000 0.5000000
```

Notice how even for matrices it is element-wise operations. However matrix multiplication does not actually work like that:

```
x <- matrix(1:12, nrow=4, ncol=3)
y <- matrix(13:24, nrow=3, ncol=4)
x %*% y</pre>
```

```
[,1] [,2] [,3] [,4]
[1,]
     218 263
               308
                    353
[2,]
     260
          314
                368
                     422
[3,]
     302
          365
                428
                     491
[4,]
     344
          416 488
                    560
```

The %*% allows us to perform matrix (you can also use the crossprod() function)

As for relational operators the same concepts of element wise apply:

```
x \leftarrow c(2,1,4,3,5)

y \leftarrow c(3,1,7,3,8)

x == y
```

[1] FALSE TRUE FALSE TRUE FALSE

```
x < y
```

[1] TRUE FALSE TRUE FALSE TRUE

```
3 %in% x
```

[1] TRUE

The relational operators apply element wise for vectors. Note the use for the %in% operate to test is an element exists in a vector

```
x <- matrix(1:12, nrow=4, ncol=3)
y <- matrix(13:24, nrow=4, ncol=3)
x == y</pre>
```

```
[,1] [,2] [,3]
[1,] FALSE FALSE FALSE
[2,] FALSE FALSE FALSE
[3,] FALSE FALSE FALSE
[4,] FALSE FALSE FALSE
```

x < y

```
[,1] [,2] [,3]
[1,] TRUE TRUE TRUE
[2,] TRUE TRUE TRUE
[3,] TRUE TRUE TRUE
[4,] TRUE TRUE TRUE
```

```
3 %in% x
```

[1] TRUE

The same goes for matrices!!

Data Frames

A dataframe is more general than a matrix in that different columns can contain different modes of data (numeric, character, and so on).

They are similar to Excel sheet or database tables, consisting of rows and columns where each column can contain different types of data. Data frames are particularly useful for working with real-world datasets.

Data frames are the most common data structure you'll deal with in R, so lets get comfortable with them!!

A data frame is created with the data.frame() function:

```
# This Creates a dataframe with column names

df <- data.frame(
   Name = c("John", "Joe", "Mary"),
   Age = c(25, 30, 35),
   City = c("New York", "San Francisco", "Boston")
)</pre>
```

In your R environment you would see the df variable with a blue drop down arrow (clicking on it will show the column names and values of your data frame), and double clicking will open up a new window that renders the dataset.

Indexing a dataframe is similar to that of a matrix:

```
df[, "Name"] # by specifying nothing for the rows you are asking for all rows
```

```
[1] "John" "Joe" "Mary"
```

```
df[1:2,] # by specifying nothing for the columns you are asking for all columns
```

```
Name Age City
1 John 25 New York
2 Joe 30 San Francisco
```

You can also use the \$ to get a specific column (this is more commonly used)

```
df$Age
```

```
[1] 25 30 35
```

Dataframes offer several methods for manipulating and transforming data. You can add or remove columns, compute new variables based on existing ones, filter rows based on specific conditions, and more.

```
df$Salary <- c(50000, 60000, 70000) # Adding a new column

df <- subset(df, select = -City) # Removing a column

df <- subset(df, Age > 25) # Filtering rows based on a condition
```

Notice the changes in df in your environment as you run the above cell.

The subset() function, n R, is used to extract a subset of a dataframe or a vector based on specified conditions. It allows you to filter rows based on logical expressions or certain criteria. It takes in the dataframe and the logical expression you want to subset by.

Dataframes can also be useful for when you read in a data table:

```
df <- read.table("TCGA_GBM_LGG_Mutations_all.csv", header=TRUE, sep=",")
head(df)</pre>
```

```
ï..Grade Project
                        Case_ID Gender Age_at_diagnosis
      LGG TCGA-LGG TCGA-DU-8164
                                  Male 51 years 108 days
2
      LGG TCGA-LGG TCGA-QH-A6CY
                                  Male 38 years 261 days
                                  Male 35 years 62 days
3
      LGG TCGA-LGG TCGA-HW-A5KM
4
      LGG TCGA-LGG TCGA-E1-A7YE Female 32 years 283 days
5
      LGG TCGA-LGG TCGA-S9-A6WG
                                  Male 31 years 187 days
6
      LGG TCGA-LGG TCGA-DB-A4X9 Female 33 years 78 days
       Primary_Diagnosis Race
                                  IDH1
                                              TP53
                                                           ATRX
                                                                       PTEN
  Oligodendroglioma, NOS white MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
1
            Mixed glioma white MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
2
         Astrocytoma, NOS white MUTATED
                                                        MUTATED NOT_MUTATED
3
                                           MUTATED
4 Astrocytoma, anaplastic white MUTATED
                                           MUTATED
                                                       MUTATED NOT_MUTATED
5 Astrocytoma, anaplastic white MUTATED
                                            MUTATED
                                                        MUTATED NOT_MUTATED
            Mixed glioma white MUTATED NOT_MUTATED
                                                        MUTATED NOT_MUTATED
         EGFR
                     CIC
                               MUC16
                                          PIK3CA
                                                          NF1
                                                                   PIK3R1
1 NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                         MUTATED NOT_MUTATED NOT_MUTATED
                 MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
3 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
4 NOT MUTATED NOT MUTATED
                             MUTATED NOT_MUTATED NOT_MUTATED
                                                                 MUTATED
5 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
6 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
       FUBP1
                     RB1
                              NOTCH1
                                            BCOR
                                                        CSMD3
                                                                  SMARCA4
     MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
2 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
3 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
4 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
5 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
6 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
       GRIN2A
                    IDH2
                                FAT4
                                          PDGFRA
1 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
2 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
3 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
4 NOT_MUTATED NOT_MUTATED
                             MUTATED NOT_MUTATED
5 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
6 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
```

This dataset, which can be found here, is from the UCIrvine Machine learning repository. Here is the description of the dataset: Gliomas are the most common primary tumors of the brain.

They can be graded as LGG (Lower-Grade Glioma) or GBM (Glioblastoma Multiforme) depending on the histological/imaging criteria. Clinical and molecular/mutation factors are also very crucial for the grading process. Molecular tests are expensive to help accurately diagnose glioma patients. In this dataset, the most frequently mutated 20 genes and 3 clinical features are considered from TCGA-LGG and TCGA-GBM brain glioma projects. The prediction task is to determine whether a patient is LGG or GBM with a given clinical and molecular/mutation features. The main objective is to find the optimal subset of mutation genes and clinical features for the glioma grading process to improve performance and reduce costs.

Here we use the read.table() function to read a .csv file into R as a dataframe. Now all the methods we just talked about are applicable here.

Factors

Factors are used in R to represent categorical or nominal data. They are especially useful when working with variables that have a limited set of distinct values or categories. Factors allow for efficient storage and manipulation of categorical data, and they play a crucial role in statistical analysis and data visualization.

You can create a factor in R using the factor() function. The factor() function takes a vector of categorical data as input and automatically identifies unique levels or categories.

```
gender <- c("Male", "Female", "Male", "Female")
(factor_gender <- factor(gender))</pre>
```

```
[1] Male Female Male Male Female Levels: Female Male
```

In this example, we create a factor called factor_gender from the vector gender, which represents the gender of individuals. The factor() function automatically identifies the unique levels, "Male" and "Female," in the gender vector and creates a factor object accordingly.

Factors have several properties and functions that make them particularly useful for handling categorical data.

```
levels(factor_gender) # This gives you ordering/levels in the factor

[1] "Female" "Male"

table(factor_gender) # Counts the frequency of each category

factor_gender
Female Male
2 3
```

In this example, we demonstrate some operations on factors. The levels() function retrieves the distinct levels or categories of the factor_gender factor. The table() function provides a frequency count of each level in the factor. Additionally, you can rename factor levels by assigning a new set of level names using the levels() function.

levels(factor_gender) <- c("M", "F", "M", "M", "F") # This renames the factor's</pre>

Factors can be ordered to represent ordinal variables with a natural ordering of levels. The ordered() function is used to create an ordered factor.

```
[1] Good Poor Excellent Fair Good
Levels: Poor < Fair < Good < Excellent
```

Essentially by ordinal we mean that they are categories with order within them.

Lists

Lists are versatile data structures in R that can hold elements of different data types, including vectors, matrices, data frames, or even other lists. Lists provide a flexible way to organize and store heterogeneous data, making them suitable for various complex data structures and analyses.

You can create a list in R using the list() function. Each element of the list can be of any data type, and they are combined into a single list object.

```
person <- list(
   name = "John Doe",
   age = 30,
   is_student = TRUE
)</pre>
```

We create a list called person using the list() function. The list contains three elements: name, age, and is_student. Each element is assigned a value, representing the name of a person, their age, and whether they are a student.

Elements within a list can be accessed using the \$ operator or the bracket ([]) notation.

```
(person_name <- person$name)
[1] "John Doe"
(person_age <- person[["age"]])</pre>
```

[1] 30

Lists offer various methods for manipulating and modifying their elements. You can add new elements, remove existing elements, or modify the values of specific elements within a list.

```
person$city <- "New York" # Adds a new element
person$is_student <- NULL # Removes an element
person$name <- "Jane Smith" # Modifies an element</pre>
```

Data Management

As R is primarily used for work with data, is this section lets learn of different methods to manipulate data. We will continue using the Glioma Grading Dataset from above

head(df)

```
Case_ID Gender Age_at_diagnosis
 ï..Grade Project
      LGG TCGA-LGG TCGA-DU-8164
                                  Male 51 years 108 days
2
      LGG TCGA-LGG TCGA-QH-A6CY
                                  Male 38 years 261 days
3
      LGG TCGA-LGG TCGA-HW-A5KM
                                  Male 35 years 62 days
      LGG TCGA-LGG TCGA-E1-A7YE Female 32 years 283 days
4
5
      LGG TCGA-LGG TCGA-S9-A6WG
                                  Male 31 years 187 days
6
      LGG TCGA-LGG TCGA-DB-A4X9 Female 33 years 78 days
       Primary_Diagnosis Race
                                  IDH1
                                              TP53
                                                          ATRX
                                                                      PTEN
  Oligodendroglioma, NOS white MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
1
2
            Mixed glioma white MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
        Astrocytoma, NOS white MUTATED
3
                                           MUTATED
                                                       MUTATED NOT MUTATED
4 Astrocytoma, anaplastic white MUTATED
                                           MUTATED
                                                       MUTATED NOT MUTATED
5 Astrocytoma, anaplastic white MUTATED
                                           MUTATED
                                                       MUTATED NOT MUTATED
            Mixed glioma white MUTATED NOT_MUTATED
                                                       MUTATED NOT_MUTATED
        EGFR
                     CIC
                               MUC16
                                          PIK3CA
                                                         NF1
                                                                  PIK3R1
1 NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                         MUTATED NOT_MUTATED NOT_MUTATED
                 MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
2 NOT MUTATED
3 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
4 NOT_MUTATED NOT_MUTATED
                             MUTATED NOT_MUTATED NOT_MUTATED
5 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
6 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
       FUBP1
                     RB1
                              NOTCH1
                                            BCOR
                                                       CSMD3
                                                                 SMARCA4
     MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
2 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
3 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
4 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
5 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
6 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
      GRIN2A
                    IDH2
                                FAT4
                                          PDGFRA
1 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
2 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
3 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
4 NOT_MUTATED NOT_MUTATED
                             MUTATED NOT MUTATED
5 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
6 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
```

Lets restructure the columns Age_at_diagnosis to have a column called AgeInYears where we convert everything in terms of years.

```
typeof(df$Age_at_diagnosis)
```

[1] "character"

Clearly it doesn't make sense for this column to be of a character type, but we need it as a numeric type.

```
years <- as.numeric(gsub(" years.*", "", df$Age_at_diagnosis))</pre>
```

Warning: NAs introduced by coercion

```
days <- as.numeric(gsub(".*years | days", "", df$Age_at_diagnosis))</pre>
```

Warning: NAs introduced by coercion

```
age_in_years <- years + days / 365
df$AgeInYears <- age_in_years
head(df)</pre>
```

```
i..Grade Project
                         Case_ID Gender Age_at_diagnosis
      LGG TCGA-LGG TCGA-DU-8164
                                   Male 51 years 108 days
1
2
       LGG TCGA-LGG TCGA-QH-A6CY
                                   Male 38 years 261 days
3
                                   Male 35 years 62 days
       LGG TCGA-LGG TCGA-HW-A5KM
4
      LGG TCGA-LGG TCGA-E1-A7YE Female 32 years 283 days
5
       LGG TCGA-LGG TCGA-S9-A6WG
                                   Male 31 years 187 days
6
       LGG TCGA-LGG TCGA-DB-A4X9 Female 33 years 78 days
       Primary_Diagnosis Race
                                   IDH1
                                               TP53
                                                           ATRX
                                                                       PTEN
  Oligodendroglioma, NOS white MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
1
             Mixed glioma white MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
2
3
         Astrocytoma, NOS white MUTATED
                                            MUTATED
                                                        MUTATED NOT MUTATED
4 Astrocytoma, anaplastic white MUTATED
                                            MUTATED
                                                        MUTATED NOT MUTATED
5 Astrocytoma, anaplastic white MUTATED
                                            MUTATED
                                                        MUTATED NOT_MUTATED
             Mixed glioma white MUTATED NOT_MUTATED
                                                        MUTATED NOT MUTATED
                      CIC
                                MUC16
                                           PIK3CA
                                                          NF1
                                                                   PIK3R1
         EGFR
1 NOT MUTATED NOT MUTATED NOT MUTATED
                                          MUTATED NOT_MUTATED NOT_MUTATED
2 NOT MUTATED
                 MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
3 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
4 NOT_MUTATED NOT_MUTATED
                              MUTATED NOT_MUTATED NOT_MUTATED
                                                                  MUTATED
5 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
6 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
        FUBP1
                      RB1
                               NOTCH1
                                             BCOR
                                                        CSMD3
                                                                  SMARCA4
      MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
2 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
3 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
4 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
5 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
6 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
       GRIN2A
                     IDH2
                                 FAT4
                                           PDGFRA AgeInYears
1 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                    51.29589
2 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                    38.71507
3 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                    35.16986
4 NOT MUTATED NOT MUTATED
                              MUTATED NOT MUTATED
                                                    32.77534
5 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
                                                    31.51233
6 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
                                                    33.21370
```

The above few lines of code will create a column called AgeInYears. Lets break it down:

In R, the gsub() function is used for pattern matching and replacement in strings. It stands for "global substitution" and is a part of the base R package. The function has the following structure: gsub(pattern, replacement, vector): - pattern: The pattern or regular expression you want to match in the string. - replacement: The replacement string that will replace the matched pattern. - vector: The input vector or string where the pattern matching and replacement will be performed.

In the above code we use the gsub() function to remove the substring "years" followed by any characters (.*) from the Age_at_diagnosis column in the dataframe. We do a similar process for the days.

Then it is nothing but a simple calculation to generate the age in years and then add it back to the df!

typeof(df\$AgeInYears)

[1] "double"

Notice how the type of the columns is now a numeric type!

Now lets use a boolean mask to create a categorical variable called Age Range. But first, what is a boolean mask: A boolean mask on data refers to a logical representation or a logical condition applied to the elements of a dataset. It is typically represented as a boolean (TRUE/FALSE) value for each element in the dataset, indicating whether a specific condition is met or not. A boolean mask can be used to filter or select specific elements or rows based on the condition it represents. By applying the boolean mask to the dataset, only the elements or rows that satisfy the condition will be included, while the others will be excluded. This allows for effective data manipulation and analysis by focusing on the desired subset of the data.

For example:

df\$AgeInYears > 60

```
[1] FALSE FALSE FALSE FALSE FALSE FALSE FALSE
                                                                                                                                                            NA FALSE FALSE
  [13] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
  [25] FALSE FALSE FALSE FALSE FALSE
                                                                                                          TRUE FALSE FALSE FALSE FALSE
  [37] FALSE FALSE FALSE FALSE
                                                                                                NA FALSE FALSE FALSE
                                                                                                                                                       TRUE FALSE FALSE
                TRUE
                               TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
                TRUE FALSE FALSE FALSE FALSE
                                                                                                       TRUE FALSE
                                                                                                                                        TRUE
                                                                                                                                                     TRUE FALSE FALSE
  [73] FALSE FALSE FALSE FALSE FALSE FALSE FALSE
                                                                                                                                        TRUE FALSE FALSE FALSE
  [85] FALSE FALSE FALSE FALSE
                                                                                           TRUE FALSE FALSE FALSE FALSE FALSE
  [97] FALSE FALSE FALSE
                                                           TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[109] FALSE FALSE FALSE FALSE
                                                                                           TRUE FALSE FALSE FALSE
                                                                                                                                                                      TRUE
[121] FALSE FALSE FALSE FALSE FALSE FALSE
                                                                                                                         TRUE
                                                                                                                                       TRUE FALSE FALSE FALSE
                TRUE FALSE FALSE
                                                             TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[145] FALSE FALSE FALSE
                                                             TRUE FALSE FALSE FALSE FALSE FALSE
[157] FALSE FALSE
                                              TRUE
                                                             TRUE FALSE
                                                                                          TRUE FALSE FALSE FALSE
                TRUE
                             TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[181] FALSE FALSE FALSE
                                                                          TRUE
                                                                                           TRUE FALSE FALSE FALSE FALSE FALSE
[193] FALSE FALSE
                                            TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[205] FALSE 
[217] FALSE FALSE FALSE FALSE
                                                                            TRUE FALSE
                                                                                                          TRUE FALSE FALSE FALSE FALSE
                                                                            TRUE FALSE
                                                                                                          TRUE
[229] FALSE FALSE FALSE
                                                                                                                         TRUE FALSE FALSE FALSE
[241] FALSE FALSE
                                            TRUE FALSE FALSE FALSE FALSE FALSE FALSE
[253] FALSE
                             TRUE FALSE FALSE FALSE FALSE FALSE
                                                                                                                                      TRUE FALSE FALSE FALSE
[265]
                TRUE FALSE FALSE FALSE FALSE
                                                                                                          TRUE FALSE FALSE FALSE FALSE
[277] FALSE FALSE
[289] FALSE 
[301] FALSE FALSE
                                              TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[313]
                TRUE FALSE
                                              TRUE FALSE FALSE FALSE
                                                                                                                       TRUE FALSE FALSE FALSE
[325] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[337] FALSE FALSE
               TRUE FALSE FALSE
                                                             TRUE FALSE
                                                                                           TRUE FALSE FALSE
                                                                                                                                      TRUE FALSE
                                                                                                                                                                    TRUE FALSE
[361] FALSE FALSE FALSE
                                                             TRUE
                                                                          TRUE FALSE FALSE FALSE FALSE FALSE FALSE
                                                           TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[373] FALSE TRUE FALSE
[385] FALSE FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE FALSE
[397] FALSE FALSE FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE FALSE
```

```
[409] FALSE FALSE FALSE FALSE FALSE
                                          TRUE FALSE FALSE
                                                            TRUE FALSE FALSE
[421] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
                                                                        TRUE
[433] FALSE FALSE FALSE FALSE
                                      NA FALSE FALSE FALSE FALSE FALSE
      TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[445]
[457] FALSE FALSE FALSE
                              TRUE FALSE FALSE FALSE FALSE FALSE
[469] FALSE FALSE
[481] FALSE FALSE FALSE FALSE
                                    TRUE
                                                 TRUE FALSE FALSE FALSE
                                          TRUE
[493] FALSE FALSE FALSE FALSE FALSE FALSE FALSE
                                                            TRUE
                                                                  TRUE
[505]
      TRUE FALSE
                  TRUE
                        TRUE
                              TRUE
                                    TRUE
                                          TRUE
                                                TRUE
                                                      TRUE FALSE FALSE
                                                                         TRUE
            TRUE FALSE
                              TRUE
                                    TRUE
                                           TRUE
                                                            TRUE FALSE FALSE
[517]
      TRUE
                        TRUE
                                                 TRUE FALSE
[529] FALSE
            TRUE FALSE FALSE
                              TRUE
                                    TRUE
                                           TRUE
                                                 TRUE FALSE FALSE FALSE
                                          TRUE
[541]
      TRUE FALSE
                  TRUE FALSE FALSE FALSE
                                                 TRUE FALSE
                                                            TRUE
                                                                   TRUE FALSE
[553]
      TRUE
            TRUE
                  TRUE
                        TRUE FALSE FALSE FALSE
                                                TRUE
                                                      TRUE FALSE FALSE
                                                                         TRUE
                                    TRUE
            TRUE
                  TRUE FALSE FALSE
                                          TRUE FALSE FALSE FALSE
                                                                   TRUE
                                                                         TRUE
[565]
      TRUE
[577]
        NA FALSE FALSE FALSE
                                    TRUE
                                           TRUE FALSE
                                                       TRUE
                                                            TRUE
                                                                   TRUE
                                                                         TRUE
[589]
      TRUE
            TRUE
                  TRUE FALSE FALSE FALSE
                                          TRUE FALSE FALSE FALSE
                                                                   TRUE FALSE
            TRUE
                  TRUE FALSE FALSE
                                    TRUE FALSE
                                                       TRUE FALSE
                                                                   TRUE
[601]
      TRUE
                                                TRUE
                                                                        TRUE
[613]
      TRUE FALSE
                  TRUE
                        TRUE FALSE FALSE
                                          TRUE
                                                 TRUE
                                                       TRUE FALSE
                                                                   TRUE FALSE
[625]
      TRUE
            TRUE
                  TRUE FALSE
                              TRUE FALSE FALSE
                                                TRUE
                                                       TRUE
                                                            TRUE FALSE
                                                                         TRUE
[637]
      TRUE
            TRUE
                  TRUE FALSE
                              TRUE FALSE
                                          TRUE FALSE FALSE
                                                             TRUE
                                                                   TRUE
                                                                         TRUE
[649] FALSE FALSE
                  TRUE
                       TRUE
                              TRUE
                                    TRUE
                                          TRUE
                                                TRUE
                                                       TRUE FALSE
                                                                   TRUE
                                                                         TRUE
      TRUE FALSE
                  TRUE FALSE
                              TRUE
                                    TRUE FALSE
                                                 TRUE FALSE
                                                             TRUE
                                                                   TRUE
                                                 TRUE
[673] FALSE FALSE
                  TRUE
                        TRUE FALSE FALSE
                                           TRUE
                                                       TRUE FALSE FALSE FALSE
      TRUE
           TRUE FALSE FALSE
                              TRUE FALSE
                                          TRUE
                                                TRUE FALSE FALSE
                                                                   TRUE
[685]
                                                                         TRUE
[697] FALSE FALSE
                  TRUE FALSE
                              TRUE FALSE FALSE FALSE
                                                            TRUE
                                                                     NA
                                                                         TRUE
[709] FALSE
            TRUE
                  TRUE
                        TRUE FALSE FALSE
                                           TRUE
                                                TRUE
                                                       TRUE FALSE FALSE FALSE
[721]
      TRUE FALSE FALSE FALSE
                              TRUE FALSE
                                           TRUE
                                                TRUE FALSE FALSE FALSE
[733] FALSE FALSE FALSE
                        TRUE
                              TRUE
                                    TRUE
                                          TRUE FALSE FALSE
                                                            TRUE
                                                                   TRUE FALSE
[745] FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
                                                                   TRUE FALSE
[757] FALSE FALSE
                  TRUE FALSE
                              TRUE FALSE
                                           TRUE
                                                 TRUE FALSE
                                                            TRUE FALSE
[769] FALSE
            TRUE
                  TRUE FALSE
                              TRUE FALSE
                                           TRUE
                                                TRUE
                                                       TRUE FALSE
                                                                   TRUE
                                                                         TRUE
[781]
      TRUE FALSE FALSE FALSE
                              TRUE
                                    TRUE
                                          TRUE FALSE
                                                       TRUE
                                                            TRUE
                                                                   TRUE FALSE
      TRUE FALSE
                              TRUE
                                           TRUE FALSE
                                                             TRUE FALSE
[793]
                    NA
                        TRUE
                                    TRUE
                                                       TRUE
[805] FALSE
            TRUE
                  TRUE FALSE
                              TRUE FALSE
                                         FALSE
                                                TRUE FALSE
                                                             TRUE FALSE
                                                                         TRUE
[817] FALSE
            TRUE FALSE
                        TRUE
                              TRUE FALSE
                                          TRUE FALSE
                                                       TRUE FALSE
                                                                   TRUE
                                                                         TRUE
[829] FALSE
            TRUE
                  TRUE FALSE
                              TRUE FALSE
                                          TRUE FALSE
                                                       TRUE
                                                            TRUE
                                                                  TRUE FALSE
[841] FALSE
            TRUE
                  TRUE FALSE FALSE FALSE
                                           TRUE FALSE FALSE FALSE
[853] FALSE TRUE
                  TRUE
                       TRUE FALSE TRUE
                                          TRUE
                                               TRUE
                                                      TRUE
                                                            TRUE
```

This logical expression is evaluated on all of the values in the AgeInYears columns and returns TRUE or FALSE depending on if the condition is met.

If you look closely at this output you will realize that there are NA values. NA means that the value at that specific data cell is missing. Why is this the case?

sum(is.na(df\$AgeInYears))

[1] 7

When running the above line you can see that there are actually 7 missing values in the AgeInYears columns. Lets take a look at these rows.

```
i..Grade Project
                          Case_ID Gender Age_at_diagnosis
10
        LGG TCGA-LGG TCGA-DU-A76K
                                    Male
                                                 87 years
42
        LGG TCGA-LGG TCGA-R8-A6YH
438
        LGG TCGA-LGG TCGA-W9-A837
                                    Male
577
        GBM TCGA-GBM TCGA-06-0744
                                    Male
                                                 67 years
672
        GBM TCGA-GBM TCGA-28-2501
707
        GBM TCGA-GBM TCGA-28-2510
795
        GBM TCGA-GBM TCGA-16-1048
        Primary_Diagnosis Race
                                       IDH1
                                                   TP53
   Oligodendroglioma, NOS white NOT_MUTATED NOT_MUTATED NOT_MUTATED
10
                                    MUTATED
                                                MUTATED
438 Oligodendroglioma, NOS white
                                    MUTATED NOT_MUTATED NOT_MUTATED
             Glioblastoma white NOT_MUTATED
                                                MUTATED NOT MUTATED
577
672
                             -- NOT MUTATED NOT MUTATED NOT MUTATED
707
                             -- NOT_MUTATED NOT_MUTATED NOT_MUTATED
795
                             -- NOT_MUTATED NOT_MUTATED NOT_MUTATED
          PTEN
                      EGFR
                                   CIC
                                             MUC16
                                                        PIK3CA
                                                                       NF1
10 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
42 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
438 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
577 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
672 NOT MUTATED NOT_MUTATED NOT_MUTATED
                                          MUTATED NOT_MUTATED NOT_MUTATED
       MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
795 NOT_MUTATED
                   MUTATED NOT_MUTATED NOT_MUTATED
                                                       MUTATED NOT MUTATED
        PIK3R1
                     FUBP1
                                   RB1
                                            NOTCH1
                                                          BCOR
10 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
42 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
438 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
577 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
672 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
707 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
795 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
                                              FAT4
                                                        PDGFRA AgeInYears
       SMARCA4
                    GRIN2A
                                  IDH2
10 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
42 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                                       NA
438 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                                       NA
577 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
                                                                       NA
672 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                                       NA
707 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                                       NA
795 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                                       NA
```

You can see that in rows 10, 42, 438, 577, 672, 707, and 795 the format of the Age_at_diagnosis is weird. It takes on values like "–" and are not specified to be any number of days old as well. To handle for these cases we can rewrite the code above as follows:

```
years <- as.numeric(gsub(" years.*", "", df$Age_at_diagnosis))</pre>
```

Warning: NAs introduced by coercion

```
days <- as.numeric(gsub(".*years | days", "", df$Age_at_diagnosis))</pre>
```

Warning: NAs introduced by coercion

```
days[is.na(days)] <- 0 # Assign 0 for missing 'days' values
years[is.na(years)] <- 0 # Assign 0 for missing 'years' values
age_in_years <- years + days / 365
df$AgeInYears <- age_in_years</pre>
```

Now if we re-run the same command we can see that there are no missing values in the AgeInYears column

```
sum(is.na(df$AgeInYears))
```

[1] 0

Lets go back to creating the AgeRange columns.

```
df$AgeRange[df$AgeInYears < 40] <- "Young"
df$AgeRange[df$AgeInYears >= 40 & df$AgeInYears < 65] <- "Old"
df$AgeRange[df$AgeInYears >= 65] <- "Elder"</pre>
```

The expressions within the brackets create a boolean mask to which we can subset the df and replace with a specific value!

```
(df$AgeRange[seq(1,10)])
```

```
[1] "Old" "Young" "Young" "Young" "Young" "Young" "Young" "Old" "Young" [10] "Elder"
```

Now, lets look at the columns of the df

```
names(df) # use the names() function to return the column names
```

```
[1] "i..Grade"
                          "Project"
                                                "Case_ID"
 [4] "Gender"
                          "Age_at_diagnosis"
                                               "Primary_Diagnosis"
 [7] "Race"
                          "IDH1"
                                               "TP53"
[10] "ATRX"
                          "PTEN"
                                               "EGFR"
[13] "CIC"
                          "MUC16"
                                               "PIK3CA"
[16] "NF1"
                                               "FUBP1"
                          "PIK3R1"
[19] "RB1"
                          "NOTCH1"
                                               "BCOR"
[22] "CSMD3"
                                               "GRIN2A"
                          "SMARCA4"
[25] "IDH2"
                          "FAT4"
                                               "PDGFRA"
[28] "AgeInYears"
                          "AgeRange"
```

Notice that one of the columns is "i..Grade", I don't know where this random character came from but it shouldn't be too hard of a fix:

```
names(df)[1] <- "Grade"
```

If you look back at the column names you can see that the changes have been made:

names(df)

```
[1] "Grade"
                          "Project"
                                                "Case_ID"
[4] "Gender"
                          "Age_at_diagnosis"
                                                "Primary_Diagnosis"
[7] "Race"
                          "IDH1"
                                                "TP53"
                          "PTEN"
                                                "EGFR"
[10] "ATRX"
[13] "CIC"
                          "MUC16"
                                                "PIK3CA"
[16] "NF1"
                          "PIK3R1"
                                                "FUBP1"
[19] "RB1"
                                                "BCOR"
                          "NOTCH1"
[22] "CSMD3"
                          "SMARCA4"
                                                "GRIN2A"
[25] "IDH2"
                          "FAT4"
                                                "PDGFRA"
[28] "AgeInYears"
                          "AgeRange"
```

Unfortunately, there are a few other columns in the df that are "improperly" formatted. For example:

unique(df\$Race)

If we look at the unique values of the Race columns, which we can do with the unique() function, you will once again see the "-" values.

So lets make so that anytime we see a value of "-" we replace it with NA so that we don't get confused.

```
df <- replace(df, df == "--", NA)</pre>
```

In the above line we use the replace() function that takes in a dataframe, a boolean mask, and the value to replace with.

unique(df\$Race)

```
[1] "white" "asian"
[3] "black or african american" NA
[5] "not reported" "american indian or alaska native"
```

Now we see that there is an NA value instead of "-" for the unique values in the Race columns

df[!complete.cases(df),]

	Grade	Project	Case_ID	Gender	Age_at_diagnosis	Primary_Diagnosis
42	LGG	TCGA-LGG	TCGA-R8-A6YH	<na></na>	<na></na>	<na></na>
438	LGG	TCGA-LGG	TCGA-W9-A837	Male	<na></na>	Oligodendroglioma, NOS
672	GBM	TCGA-GBM	TCGA-28-2501	<na></na>	<na></na>	<na></na>
707	GBM	TCGA-GBM	TCGA-28-2510	<na></na>	<na></na>	<na></na>

```
GBM TCGA-GBM TCGA-16-1048
                                                   <NA>
                                                                          <NA>
                                  <NA>
                 TDH1
                            TP53
                                        ATRX
                                                    PTEN
     Race
                                                                F.GFR.
             {\tt MUTATED}
                         MUTATED
42
     <NA>
                                     MUTATED NOT MUTATED NOT MUTATED
             MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
438 white
672
    <NA> NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
     <NA> NOT MUTATED NOT MUTATED NOT MUTATED
                                                 MUTATED NOT MUTATED
     <NA> NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
                                                             MUTATED
                     MUC16
                                PIK3CA
                                               NF1
                                                        PIK3R1
42 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
438 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
672 NOT_MUTATED
                   MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
707 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
795 NOT_MUTATED NOT_MUTATED
                               MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                  BCOR
            RB1
                    NOTCH1
                                             CSMD3
                                                        SMARCA4
42 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
438 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
672 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
707 NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED NOT MUTATED
795 NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED NOT_MUTATED
                      FAT4
                                PDGFRA AgeInYears AgeRange
42 NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                     Young
438 NOT MUTATED NOT MUTATED NOT MUTATED
                                                     Young
672 NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                0
                                                     Young
707 NOT MUTATED NOT MUTATED NOT MUTATED
                                                0
                                                     Young
795 NOT_MUTATED NOT_MUTATED NOT_MUTATED
                                                      Young
```

Here we use the complete.cases() function, which returns a logical vector indicating which rows are complete (no NAs) or incomplete (contain at least one NA). By negating the result with !, we get a boolean mask for rows with at least one NA value.

The output includes all the rows with at least one missing values, thankfully there aren't that many.

When cleaning and manipulating a dataset, the way you deal with missing values can be very important. This area of missing value imputation has various strategies from imputing with the mean or median, to using machine learning models to estimate and replace missing values. This is however outside the scope of this tutorial, but here is a good link to learn more!

For now we will just go ahead and delete all these row with a missing value using the na.omit() function:

```
df <- na.omit(df)</pre>
```

Lets look at the data types of all the columns int eh df

```
str(df)
```

```
'data.frame':
              857 obs. of
                           29 variables:
                         "LGG" "LGG" "LGG" ...
$ Grade
                   : chr
$ Project
                         "TCGA-LGG" "TCGA-LGG" "TCGA-LGG" ...
                   : chr
                         "TCGA-DU-8164" "TCGA-QH-A6CY" "TCGA-HW-A5KM" "TCGA-E1-A7YE" ...
$ Case_ID
                   : chr
                         "Male" "Male" "Female" ...
$ Gender
                    chr
                         "51 years 108 days" "38 years 261 days" "35 years 62 days" "32 years 283 day
$ Age_at_diagnosis : chr
                         "Oligodendroglioma, NOS" "Mixed glioma" "Astrocytoma, NOS" "Astrocytoma, ana
$ Primary_Diagnosis: chr
                         "white" "white" "white" ...
$ Race
                   : chr
                         "MUTATED" "MUTATED" "MUTATED" ...
$ IDH1
                   : chr
```

```
$ TP53
              : chr
                      "NOT_MUTATED" "NOT_MUTATED" "MUTATED" "MUTATED" ...
$ ATRX
               : chr
                      "NOT_MUTATED" "NOT_MUTATED" "MUTATED" "MUTATED" ...
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ PTEN
               : chr
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ EGFR
               : chr
                      "NOT_MUTATED" "MUTATED" "NOT_MUTATED" ...
$ CIC
               : chr
$ MUC16
                      "NOT MUTATED" "NOT MUTATED" "NOT MUTATED" ...
               : chr
                      "MUTATED" "NOT MUTATED" "NOT MUTATED" "NOT MUTATED" ...
$ PIK3CA
              : chr
                      "NOT MUTATED" "NOT MUTATED" "NOT MUTATED" ...
$ NF1
                : chr
              : chr
$ PIK3R1
                      "NOT MUTATED" "NOT MUTATED" "NOT MUTATED" "MUTATED" ...
                      "MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ FUBP1
              : chr
$ RB1
               : chr
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ NOTCH1
               : chr
              : chr
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ BCOR
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ CSMD3
               : chr
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ SMARCA4
               : chr
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ GRIN2A
               : chr
$ IDH2
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
               : chr
                      "NOT MUTATED" "NOT MUTATED" "NOT MUTATED" ...
$ FAT4
               : chr
                      "NOT_MUTATED" "NOT_MUTATED" "NOT_MUTATED" ...
$ PDGFRA
               : chr
$ AgeInYears
                : num 51.3 38.7 35.2 32.8 31.5 ...
$ AgeRange
                : chr "Old" "Young" "Young" "Young" ...
- attr(*, "na.action")= 'omit' Named int [1:5] 42 438 672 707 795
..- attr(*, "names")= chr [1:5] "42" "438" "672" "707" ...
```

clearly, the columns labelled as "chr" should be factors, so lets convert them!

```
categorical_cols <- names(df) [names(df) != "AgeInYears"]
df[categorical_cols] <- lapply(df[categorical_cols], factor)
str(df)</pre>
```

```
'data.frame': 857 obs. of 29 variables:
$ Grade
                  : Factor w/ 2 levels "GBM", "LGG": 2 2 2 2 2 2 2 2 2 ...
                  : Factor w/ 2 levels "TCGA-GBM", "TCGA-LGG": 2 2 2 2 2 2 2 2 2 ...
$ Project
                  : Factor w/ 857 levels "TCGA-02-0003",...: 478 730 690 516 772 386 705 564 657 495 .
$ Case_ID
                   : Factor w/ 2 levels "Female", "Male": 2 2 2 1 2 1 1 1 1 2 ...
$ Gender
$ Age at diagnosis : Factor w/ 837 levels "14 years 154 days",..: 410 223 174 126 104 148 177 315 142
$ Primary_Diagnosis: Factor w/ 6 levels "Astrocytoma, anaplastic",..: 6 4 2 1 1 4 5 4 6 6 ...
                  : Factor w/ 5 levels "american indian or alaska native",...: 5 5 5 5 5 5 5 5 5 5 ...
$ Race
$ IDH1
                  : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 1 1 1 1 1 1 1 1 2 ...
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 1 1 1 2 1 1 1 2 ...
$ TP53
                  : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 1 1 1 1 2 1 1 2 ...
$ ATRX
$ PTEN
                  : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 ...
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 ...
$ EGFR
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 1 2 2 2 2 2 2 2 2 ...
$ CIC
                  : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 1 2 2 2 2 2 2 ...
$ MUC16
                  : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 1 2 2 2 2 2 2 2 2 2 ...
$ PIK3CA
$ NF1
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 1 2 2 2 2 2 ...
$ PIK3R1
$ FUBP1
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 1 2 2 2 2 2 2 2 2 2 ...
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
$ RB1
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
$ NOTCH1
                 : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
$ BCOR
              : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 ...
$ CSMD3
```

```
$ SMARCA4
                   : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
$ GRIN2A
                   : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
$ IDH2
                   : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
$ FAT4
                   : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 1 2 2 2 2 2 2 ...
$ PDGFRA
                   : Factor w/ 2 levels "MUTATED", "NOT_MUTATED": 2 2 2 2 2 2 2 2 2 2 ...
$ AgeInYears
                   : num 51.3 38.7 35.2 32.8 31.5 ...
                   : Factor w/ 3 levels "Elder", "Old", ...: 2 3 3 3 3 3 3 2 3 1 ...
$ AgeRange
- attr(*, "na.action")= 'omit' Named int [1:5] 42 438 672 707 795
 ..- attr(*, "names")= chr [1:5] "42" "438" "672" "707" ...
```

Here we use boolean masking, yet again, to get all the columns, but the AgeInYear one, and then use the lapply() function to apply the factor() function to all of the categorical columns.

Basic Statistical Functions

In this section we will cover some basic functions you can use to learn more about your data.

The mean() and median() functions are used to calculate the mean and median of a vector:

```
(mean(df$AgeInYears))
```

[1] 50.92447

```
(median(df$AgeInYears))
```

[1] 51.55068

The mean and median are pretty close to each other indicating that there are not that many outliers in the dataset

We can also use the sd() for standard deviation or var() for variance to understand the spread of our dataset.

```
(sd(df$AgeInYears))
```

[1] 15.7328

```
(var(df$AgeInYears))
```

[1] 247.5209

Here are a few more functions to let you learn more about your data:

```
(sum(df$AgeInYears)) # sum of all the values in the column
```

[1] 43642.27

```
(min(df$AgeInYears)) # minimum of all the values in the column
```

[1] 14.42192

```
(max(df$AgeInYears)) # maximum of all the values in the column
```

[1] 89.28767

```
(range(df$AgeInYears)) # the minimum and the maximum of the column
```

```
[1] 14.42192 89.28767
```

The quantile() function is also pretty useful. For a probability (p) 0 to 1, quantile(x,probs=p) returns a number Q such that 100p% of the sample are less than Q.

```
(quantile(df$AgeInYears, probs = 0.3))
```

30% 40.11123

30% of the samples in the dataset are less the 40.11 years old.

To get these results of all the columns we can use the summary() function. This function provides a summary of the distribution of each variable in a dataframe or a numeric vector. It includes the minimum, 1st quartile, median, mean, 3rd quartile, and maximum values. And for categorical (factor) variables it will return the counts for all of the categories.

summary(df)

```
Grade
                                  Case_ID
                                                 Gender
              Project
GBM:360
                                             Female:359
          TCGA-GBM:360
                          TCGA-02-0003: 1
LGG:497
          TCGA-LGG:497
                          TCGA-02-0033: 1
                                             Male :498
                          TCGA-02-0047:
                          TCGA-02-0055: 1
                          TCGA-02-2466:
                          TCGA-02-2470: 1
                          (Other)
                                      :851
                                               Primary Diagnosis
         Age_at_diagnosis
64 years 298 days: 3
                           Astrocytoma, anaplastic
                                                         :129
30 years 32 days : 2
                           Astrocytoma, NOS
                                                         : 58
31 years 187 days: 2
                           Glioblastoma
                                                         :360
35 years 68 days : 2
                           Mixed glioma
                                                         :128
38 years 13 days :
                           Oligodendroglioma, anaplastic: 75
38 years 203 days:
                    2
                           Oligodendroglioma, NOS
                                                         :107
(Other)
                  :844
                                                                    TP53
                               Race
                                                  IDH1
american indian or alaska native: 1
                                        MUTATED
                                                    :412
                                                           MUTATED
                                                                      :353
asian
                                 : 14
                                        NOT_MUTATED: 445
                                                           NOT_MUTATED: 504
black or african american
                                 : 59
not reported
                                 : 18
                                 :765
white
         ATRX
                            PTEN
                                              EGFR
                                                                 CIC
MUTATED
                                     MUTATED
           :219
                  MUTATED
                              :143
                                                 :113
                                                        MUTATED
                                                                   :114
```

NOT MUTATED:638	NOT MUTATED:714	NOT MUTATED:744	NOT MUTATED:743

MUC16	PIK3CA	NF1	PIK3R1
MUTATED : 99	MUTATED : 76	MUTATED : 69	MUTATED : 57
NOT_MUTATED:758	NOT_MUTATED:781	NOT_MUTATED:788	NOT_MUTATED:800

F	UBP1		RB1	N	OTCH1		BCOR
MUTATED	: 47	MUTATED	: 41	MUTATED	: 38	MUTATED	: 29
NOT_MUTAT	ED:810	NOT_MUTAT	ED:816	NOT_MUTAT	ED:819	NOT_MUTA	TED:828

C	SMD3	SM	ARCA4	G	RIN2A		IDH2
MUTATED	: 28	MUTATED	: 28	MUTATED	: 27	MUTATED	: 23
NOT_MUTAT	ED:829	NOT_MUTAT	ED:829	NOT_MUTAT	ED:830	NOT_MUTAT	ΓED:834

FAT4	PDGFRA	${\tt AgeInYears}$	AgeRange
MUTATED : 23	MUTATED : 22	Min. :14.42	Elder:177
NOT_MUTATED:834	NOT_MUTATED:835	1st Qu.:38.02	Old :424
		Median :51.55	Young:256
		Mean :50.92	
		3rd Qu.:62.77	
		Max. :89.29	

Visualizations

R's graphing capabilities enable users to transform data into informative and visually appealing representations, such as bar charts, pie charts, histograms, and more. In this section we will learn some basic charting abilities within R

Bar Plot

- 1) Bar plots show the frequency of a categorical variable
- 2) You should only use this if the number of categories are low

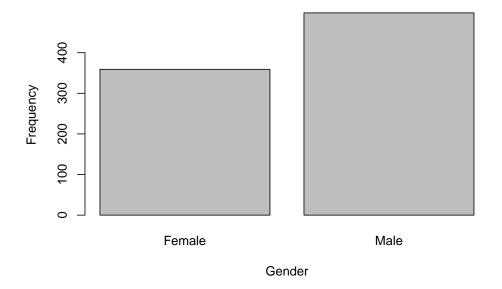
We use the barplot(vector) function to generate a bar plot where vector is the vector of frequencies.

```
(counts <- table(df$Gender))

Female Male
359 498

barplot(counts,
    main="Counts of Male and Female in the Study", #title or the chart
    xlab="Gender", # x-axis label
    ylab="Frequency") # y-axis label</pre>
```

Counts of Male and Female in the Study



This chart shows that there are more Male participants that female participants in the study. We can create a little more for a fancier bar plot called a stacked bar plot:

```
(counts <- table(df$Gender, df$Grade))</pre>
```

```
GBM LGG
Female 138 221
Male 222 276
```



This stacked bar plot shows us which proportion of the Glioma Grading types are Male and Female

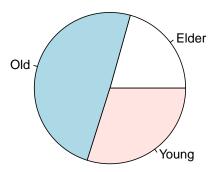
Pie Charts

1) These kind of charts are used to show proportions of different categories out of 100%.

We can use the pie(vector) function to generate a pie chart in which vector is the sequence of variables.

```
freq <- table(df$AgeRange)
pie(freq, main="Age Range")</pre>
```

Age Range



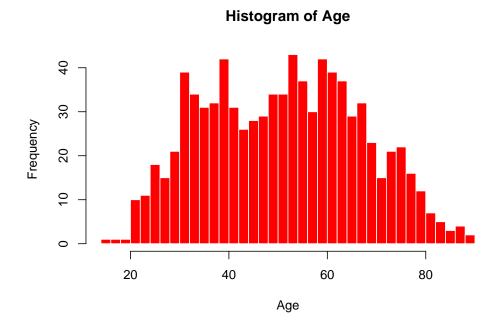
Clearly the majority of the participants in the dataset are "Old".

${\bf Histograms}$

1) A histogram displays the distribution of a continuous variable by dividing the range of values into a number of bins (groups of data points) on the x-axis and displaying the frequency of each bin on the y-axis.

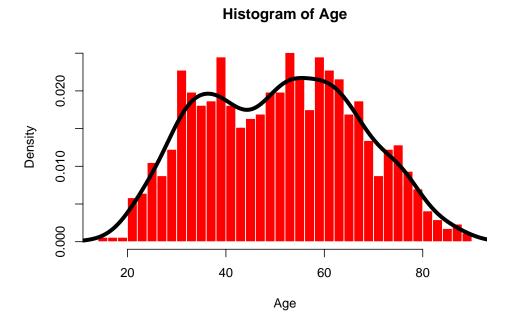
We use the hist(vector) function to generate histograms which take as an input a vector of numbers.

```
hist(df$AgeInYears,
    breaks=50,
    main="Histogram of Age",
    col="red",
    border="white",
    xlab = "Age")
```



While histograms show the distribution of a column, to see a better representation of the distribution we can use the kernel density plot, via the density() function.

A kernel density plot works by estimating the density of data points at different values along the variable's range.

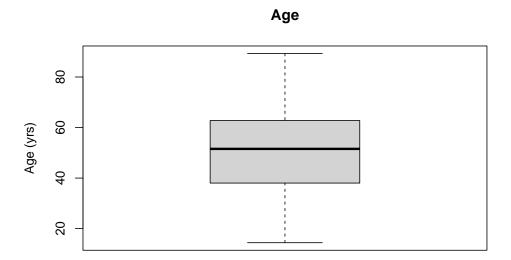


The data is bi-modally distributed.

Box & Whisker plots

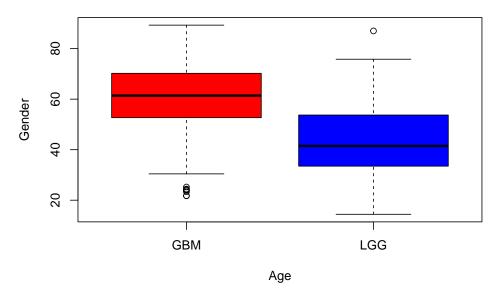
1) These plot can also describe the distribution of a continuous variable (column) by giving a visual of the 5 number summary (minimum, lower quartile (25th percentile), median (50th percentile), upper quartile (75th percentile), and maximum).

We use the boxplot(vector) to display a box and whisker plot:



You can also create a box plot based on a group:

Age and Gender



The ages of the participants with with GBM seem to be older than those with LGG.

AgeInYears ~ Gender is a formula notation used to specify a relationship or model between the dependent variable AgeInYears and the independent variable Gender in a statistical analysis or data modeling context.

The tilde (~) operator in the formula notation separates the dependent variable from the independent variable(s). In this case, AgeInYears is the dependent variable, and Gender is the independent variable.

When using this formula notation, it implies that you want to study or model the relationship between AgeInYears and Gender. It suggests that you are interested in understanding how the variable Gender might influence or be associated with the variable AgeInYears.

Conditional Statements, Loops, and Functions in R

Conditional Statements

Conditional statements allow you to execute different blocks of code based on certain conditions. In R, common conditional statements include if, if-else, if-else if-else, and the switch statement. These statements evaluate conditions and perform specific actions accordingly.

```
x <- 10
if (x > 0) {
  print("x is positive")
}
```

[1] "x is positive"

```
x <- -5
if (x > 0) {
  print("x is positive")
} else {
  print("x is non-positive")
}
```

[1] "x is non-positive"

```
x <- 0
if (x > 0) {
   print("x is positive")
} else if (x < 0) {
   print("x is negative")
} else {
   print("x is zero")
}</pre>
```

[1] "x is zero"

```
"Saturday",
"Sunday")
print(weekday)
```

[1] "Tuesday"

The if, if-else, and if-else if-else statements evaluate conditions and execute the corresponding code blocks based on the results, similar to the previous explanation.

The switch statement evaluates an expression (day in the example) and matches it to different cases. It executes the code block corresponding to the matched case. In this example, if day is 2, it assigns "Tuesday" to the weekday variable.

NOTE: We are using the print() function to also display the output.

Loops

Loops are used to repeatedly execute a block of code. R supports for loops and while loops, providing flexibility for different iteration scenarios.

```
for (i in 1:5) {
  print(paste("For loop Iteration", i))
[1] "For loop Iteration 1"
[1] "For loop Iteration 2"
[1] "For loop Iteration 3"
[1] "For loop Iteration 4"
[1] "For loop Iteration 5"
i <- 1
while (i <= 5) {
  print(paste("While loop Iteration", i))
  i <- i + 1
[1] "While loop Iteration 1"
[1] "While loop Iteration 2"
[1] "While loop Iteration 3"
[1] "While loop Iteration 4"
[1] "While loop Iteration 5"
```

The for loop iterates over a sequence (1:5 in the example), executing the code block for each iteration. It prints "For loop Iteration 1" through "For loop Iteration 5".

The while loop continues executing the code block as long as the condition remains TRUE. It increments the i variable within the loop, and the loop terminates when i becomes greater than 5.

Functions

Functions in R allow you to encapsulate reusable blocks of code, making your programs more modular and organized. You can define your own functions or use built-in functions from packages.

```
calculate_sum <- function(a, b) {
  sum <- a + b
  return(sum)
}

result <- calculate_sum(3, 5)
print(result)</pre>
```

[1] 8

Functions can be used to perform specific tasks, enhance code reusability, and promote efficient programming practices.

Working with Packages & Libraries within R

R's package ecosystem is a vast collection of pre-built tools, functions, and libraries contributed by the R community. These packages extend the functionality of the base R system and offer specialized tools for various tasks in data analysis, visualization, machine learning, and more. The package ecosystem in R is one of its key strengths, providing users with a wide range of options to efficiently perform data analysis tasks.

For this section we will be using the famous seqinr package. The seqinr library is a comprehensive R package specifically designed for the analysis and manipulation of biological sequence data, including DNA, RNA, and protein sequences. It offers a wide range of functionalities that allows bioinformaticians to perform various sequence-related tasks, such as computing sequence statistics (e.g., length, GC content), performing pairwise sequence alignments, and searching for sequence motifs

To make use of specific packages, you need to install them first. The install.packages() function in R allows you to download and install packages from the comprehensive CRAN (Comprehensive R Archive Network) repository or other repositories. For example:

```
# install.packages("seqinr"), run this to install the package
```

Once a package is installed, you can load it into your R session using the library() or require() functions. This makes the package's functions, data sets, and other resources available for use in your code. For example:

```
library(seqinr)
```

```
Warning: package 'seqinr' was built under R version 4.1.3
```

The seqinr comes with built-in datasets that allow us to practice without the need for external data. Let's explore one of these datasets, the fasta dataset, which contains DNA sequences for the OPA protein-coding genes:

Lets load in this dataset:

```
data(fasta)
DNA_SEQ <- fasta[3]
first_seq <- DNA_SEQ$seq[[1]][1]
second_seq <- DNA_SEQ$seq[[2]][1]</pre>
```

Here we use the translate() function to translate the sequence to its amino acid sequence.

```
protein_sequence <- translate(unlist(strsplit(first_seq, "")))
protein_sequence</pre>
```

```
[1] "M" "M" "S" "A" "E" "P" "P" "S" "S" "Q" "P" "Y" "I" "S" "D" "V" "L" "R"
 [19] "R" "Y" "Q" "L" "E" "R" "F" "Q" "C" "A" "F" "A" "S" "S" "M" "T" "I" "K"
 [37] "D" "L" "L" "A" "L" "Q" "P" "E" "D" "F" "N" "R" "Y" "G" "V" "V" "E" "A"
 [55] "M" "D" "I" "L" "R" "L" "R" "D" "A" "I" "E" "Y" "I" "K" "A" "N" "P" "L"
 [73] "P" "A" "S" "R" "S" "G" "S" "D" "V" "L" "D" "N" "D" "G" "D" "G" "D" "G"
 [91] "D" "D" "S" "T" "P" "E" "G" "K" "E" "G" "C" "S" "T" "E" "R" "R" "R" "Q"
[109] "Y" "T" "A" "R" "G" "T" "T" "V" "L" "C" "R" "S" "T" "D" "T" "A" "E" "E"
[127] "V" "K" "R" "K" "S" "R" "I" "L" "V" "A" "I" "R" "K" "R" "P" "L" "S" "A"
[145] "G" "E" "Q" "T" "N" "G" "F" "T" "D" "I" "M" "D" "A" "D" "N" "S" "G" "E"
[163] "I" "V" "L" "K" "E" "P" "K" "V" "K" "V" "D" "L" "R" "K" "Y" "T" "H" "V"
[181] "H" "R" "F" "F" "F" "D" "E" "V" "F" "D" "E" "A" "C" "D" "N" "V" "D" "V"
[199] "Y" "N" "R" "A" "A" "R" "A" "L" "I" "D" "T" "V" "F" "D" "G" "G" "C" "A"
[217] "T" "C" "F" "A" "Y" "G" "O" "T" "G" "S" "G" "K" "T" "H" "T" "M" "L" "G"
[235] "K" "G" "P" "E" "P" "G" "L" "Y" "A" "L" "A" "A" "K" "D" "M" "F" "D" "R"
[253] "L" "T" "S" "D" "T" "R" "I" "V" "V" "S" "F" "Y" "E" "I" "Y" "S" "G" "K"
[271] "L" "F" "D" "L" "L" "N" "G" "R" "R" "P" "L" "R" "A" "L" "E" "D" "D" "K"
[289] "G" "R" "V" "N" "I" "R" "G" "L" "T" "E" "H" "C" "S" "T" "S" "V" "E" "D"
[307] "L" "M" "T" "I" "I" "D" "Q" "G" "S" "G" "V" "R" "S" "C" "G" "S" "T" "G"
[325] "A" "N" "D" "T" "S" "S" "R" "S" "H" "A" "I" "L" "E" "I" "K" "L" "K" "A"
[343] "K" "R" "T" "S" "K" "Q" "S" "G" "K" "F" "T" "F" "I" "D" "L" "A" "G" "S"
[361] "E" "R" "G" "A" "D" "T" "V" "D" "C" "A" "R" "Q" "T" "R" "L" "E" "G" "A"
[379] "E" "I" "N" "K" "S" "L" "L" "A" "L" "K" "E" "C" "I" "R" "F" "L" "D" "Q"
[397] "N" "R" "K" "H" "V" "P" "F" "R" "G" "S" "K" "L" "T" "E" "V" "L" "R" "D"
[415] "S" "F" "I" "G" "N" "C" "R" "T" "V" "M" "I" "G" "A" "V" "S" "P" "S" "N"
[433] "N" "N" "A" "E" "H" "T" "L" "N" "T" "L" "R" "Y" "A" "D" "R" "V" "K" "E"
[451] "L" "K" "R" "N" "A" "T" "E" "R" "R" "T" "V" "C" "M" "P" "D" "D" "Q" "E"
[469] "E" "A" "F" "F" "D" "T" "T" "E" "S" "R" "P" "P" "S" "R" "R" "T" "T" "T"
[487] "R" "L" "S" "T" "A" "A" "P" "L" "F" "S" "G" "S" "S" "T" "A" "A" "P" "A"
[505] "L" "R" "S" "T" "L" "L" "S" "S" "R" "S" "V" "N" "T" "L" "S" "P" "S" "S"
[523] "Q" "A" "K" "S" "T" "L" "V" "T" "P" "K" "P" "P" "S" "R" "D" "R" "T" "P"
[541] "D" "M" "V" "C" "T" "K" "R" "P" "R" "D" "S" "D" "R" "R" "S" "G" "E" "D" "E"
[559] "V" "V" "A" "R" "P" "S" "G" "R" "P" "S" "F" "K" "R" "F" "E" "S" "G" "A"
[577] "E" "L" "V" "A" "A" "O" "R" "S" "R" "V" "I" "D" "Q" "Y" "N" "A" "Y" "L"
[595] "E" "T" "D" "M" "N" "C" "I" "K" "E" "E" "Y" "Q" "V" "K" "Y" "D" "A" "E"
[613] "Q" "M" "N" "A" "N" "T" "R" "S" "F" "V" "E" "R" "A" "R" "L" "L" "V" "S"
[631] "E" "K" "R" "R" "A" "M" "E" "S" "F" "L" "T" "Q" "L" "E" "E" "L" "D" "K"
[649] "I" "A" "Q" "Q" "V" "A" "D" "I" "T" "A" "F" "Q" "Q" "H" "L" "P" "P" "T"
```