R Programming Basics – Hands-On Practical

Igor Ruiz de los Mozos

Programme: Master's in Biomedical Engineering • **Module:** Advanced Bioinformatics
Lesson R Fundamentals, Data Wrangling & Visualisation

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1 Overview & Learning Objectives

Introduction

This practical introduces the foundational concepts of R programming and its application in bioinformatics. R is a powerful language for statistical computing and data visualization, widely used in biomedical research.

Goals

By the end of this session, you will be able to: - Navigate the RStudio interface and understand its key components. - Write and execute basic R code for data manipulation and visualization. - Use **dplyr** verbs to filter, summarize, and join datasets. - Reshape data using **tidyr** for better analysis. - Create a variety of plots using **ggplot2**, including histograms, scatter plots, and heatmaps. - Apply these skills to analyze a realistic dataset (`biomed_data.csv`) containing information on 200 patients.

Why Learn R?

R is an essential tool for bioinformatics due to its flexibility, extensive libraries, and active community. It allows researchers to handle large datasets, perform complex statistical analyses, and create publication-quality visualizations.

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2 The RStudio Interface

Pane	Purpose	Tip
Source/Editor (top-left)	Write & save scripts (`.R`, `.Rmd`)	`Ctrl/Cmd + Shift + C` toggles comments
Console (bottom-left)	Run commands interactively	`↑/↓` scrolls through history
Environment / History (top-right)	View objects & past commands	Click to remove objects
Files / Plots / Packages / Help	File nav, graphics, install pkgs, docs	Click a plot → *Export* to save

> **Tip:** Use the shortcut `Ctrl + Enter` (Windows) or `Cmd + Enter` (Mac) to run a line of code from the Source Pane in the Console.

```r
# Set your working directory once per session
getwd()
```

```
[1] "/home/rstudio"
```

```r
setwd("/home/rstudio")


# Install required libraries once:

install.packages(c("tidyverse", "viridis", "GGally"))
```

```
Installing packages into '/usr/local/lib/R/site-library'
(as 'lib' is unspecified)
```

3 R Programming Basics

Writing Your First R Code

Let's start with some simple R commands. Open the R Console and try the following:

3.1 Console vs Editor

- Console for quick commands.
- Editor for scripts.
- Execute lines with Ctrl/Cmd + Enter.

```r
# Basic arithmetic
2 + 2  # Addition
```

```
[1] 4
```

```r
5 * 3  # Multiplication
```

```
[1] 15
```

```
10 / 2 # Division
```

```
[1] 5
```

Explanation Comments: Lines starting with # are comments and are ignored by R. Assignment: The <- operator assigns values to variables. You can also use = but <- is preferred in R. Execution: Type the code in the Console or write it in a script and run it. Exercise: Try assigning different values to x and y and observe how z changes.

3.2 Variable Assignment & Arithmetic

```
x <- 10     # arrow assignment
y = 5       # equals also valid

x + y       # addition
```

```
[1] 15
```

```
x * y       # multiplication
```

```
[1] 50
```

```
x ^ 2       # exponentiation
```

```
[1] 100
```

```
x > y       # logical test
```

```
[1] TRUE
```

Explanation Comments: Lines starting with # are comments and are ignored by R. Assignment: The <- operator assigns values to variables. You can also use = but <- is preferred in R for clarity and consistency. Arithmetic Operations: R supports basic arithmetic operations like addition (+), multiplication (*), and exponentiation (^). Logical Tests: Logical operators like > return TRUE or FALSE based on the comparison. Exercise: Assign different values to x and y and observe how the results of x + y and x * y change. Try other logical operators like <, ==, and != to compare x and y.

3.3 Data Types & Coercion

```
my_numeric <- 42.0
my_char    <- "Hello"
my_logical <- TRUE

class(my_numeric)   # "numeric"
```

```
[1] "numeric"
```

```
as.character(my_numeric)  # "42"
```

```
[1] "42"
```

Explanation Data Types: R has several basic data types, including: numeric: Numbers with or without decimals. character: Text or strings. logical: Boolean values (TRUE or FALSE). Type Coercion: Functions like as.character() can convert one data type to another. For example, a numeric value can be converted to a character string. Exercise: Create variables of different data types (e.g., integer, factor) and use the class() function to check their types. Try coercing a character string to numeric using as.numeric() and observe the result.

3.4 Exploring Objects

```
v <- c(1,2,3,4,5)
str(v)         # structure
```

```
 num [1:5] 1 2 3 4 5
```

```
summary(v)     # summary stats
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max. 
      1       2       3       3       4       5 
```

```
length(v)      # 5
```

```
[1] 5
```

Explanation Vectors: The `c()` function combines values into a vector. Vectors are one of the most basic data structures in R. Functions: R provides built-in functions like `length()`, `sum()`, and `mean()` to operate on vectors. Exercise: Create a vector with at least 10 elements and calculate its sum, mean, and length. Try creating a character vector (e.g., c("A", "B", "C")) and observe what happens when you use sum() or mean() on it.

4 Exploring Objects & Structures

Vectors

```
v <- c(10, 20, 30, 40, 50)

v[1]        # Access the first element
```

```
[1] 10
```

```
v[2:4]      # Access elements from index 2 to 4
```

```
[1] 20 30 40
```

```
v[c(1, 5)] # Access the 1st and 5th elements
```

```
[1] 10 50
```

```
v[v > 25]  # Subset elements greater than 25
```

```
[1] 30 40 50
```

Explanation Indexing: Use square brackets `[]` to access specific elements of a vector. Indexing in R starts at 1 (not 0). Subsetting: You can subset vectors using logical conditions (e.g., v > 25). Exercise: Create a vector of your choice and practice accessing individual elements and ranges. Use logical conditions to subset elements based on specific criteria (e.g., values less than 15).

Matrices

```r
# Create a 3x3 matrix
m <- matrix(1:9, nrow = 3, byrow = TRUE)

m[2, 3]         # Access the element in the 2nd row, 3rd column
```

```
[1] 6
```

```r
dim(m)          # Get the dimensions of the matrix (rows and columns)
```

```
[1] 3 3
```

```r
m[1, ]          # Access the entire first row
```

```
[1] 1 2 3
```

```r
m[, 2]          # Access the entire second column
```

```
[1] 2 5 8
```

Explanation Matrix Creation: The `matrix()` function creates a matrix. The nrow argument specifies the number of rows, and byrow = TRUE fills the matrix row-wise. Indexing: Use `[row, column]` to access specific elements. Leaving the row or column blank (e.g., `[1, ]`) selects all elements in that dimension. Dimensions: The `dim()` function returns the dimensions of the matrix as a vector (number of rows and columns). Exercise: Create a 4x4 matrix with numbers from 1 to 16. Access the element in the 3rd row and 4th column. Extract the 2nd row and the 3rd column separately. Try adding two matrices of the same dimensions.

Lists

```r
# Create a list containing a vector and a matrix
my_list <- list(a = v, b = m)

my_list$a      # Access the vector by name
```

```
[1] 10 20 30 40 50
```

```
my_list$b      # Access the matrix by name
```

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

```
my_list[[1]]   # Access the first element of the list (vector)
```

```
[1] 10 20 30 40 50
```

```
my_list[[2]][2, 3]  # Access the 2nd row, 3rd column of the matrix in the list
```

```
[1] 6
```

Explanation Lists: A list is a flexible data structure that can contain elements of different types (e.g., vectors, matrices, data frames). Accessing Elements: Use `$` to access elements by name or `[[ ]]` to access elements by position. You can also combine indexing to access specific parts of nested elements. Exercise: Create a list containing a numeric vector, a character vector, and a matrix. Access each element of the list using `$` and `[[ ]]`. Modify one of the elements in the list (e.g., change a value in the matrix). Add a new element to the list (e.g., a logical vector).

Data Frames

```
# Create a data frame with 3 columns 10 rows
df <- data.frame(gene = c("G1","G2","G3","G4","G5","G6","G7","G8","G9","G10"),
                 exp =  c(1.2, 3.4, 5.6, 7.8, 9.0, 2.3, 4.5, 6.7, 8.9, 10.1),
                 group = c("A", "B", "A", "B", "A", "B", "A", "B", "A", "B"),
                 stringsAsFactors = FALSE)
str(df)
```

```
'data.frame':   10 obs. of  3 variables:
 $ gene : chr  "G1" "G2" "G3" "G4" ...
 $ exp  : num  1.2 3.4 5.6 7.8 9 2.3 4.5 6.7 8.9 10.1
 $ group: chr  "A" "B" "A" "B" ...
```

```r
summary(df)
```

```
     gene                exp            group          
 Length:10          Min.   : 1.200   Length:10         
 Class :character   1st Qu.: 3.675   Class :character  
 Mode  :character   Median : 6.150   Mode  :character  
                    Mean   : 5.950                     
                    3rd Qu.: 8.625                     
                    Max.   :10.100                     
```

```r
# Accessing data frame elements
# Accessing data frame elements
df$gene        # Access the "gene" column
```

```
 [1] "G1"  "G2"  "G3"  "G4"  "G5"  "G6"  "G7"  "G8"  "G9"  "G10"
```

```r
df[1, ]       # Access the first row
```

```
  gene exp group
1   G1 1.2     A
```

```r
df[ , 2]      # Access the second column
```

```
 [1]  1.2  3.4  5.6  7.8  9.0  2.3  4.5  6.7  8.9 10.1
```

```r
df[df$exp > 5, ] # Subset rows where exp is greater than 5
```

```
   gene  exp group
3    G3  5.6     A
4    G4  7.8     B
5    G5  9.0     A
8    G8  6.7     B
9    G9  8.9     A
10  G10 10.1     B
```

```r
df[df$group == "A", ] # Subset rows where group is "A"
```

```
  gene exp group
1   G1 1.2     A
3   G3 5.6     A
5   G5 9.0     A
7   G7 4.5     A
9   G9 8.9     A
```

```
df[df$exp > 5 & df$group == "A", ] # Subset rows where exp is greater than 5 and group is "A
```

```
  gene exp group
3   G3 5.6     A
5   G5 9.0     A
9   G9 8.9     A
```

```
# Adding a new column
df$Pass <- ifelse(df$exp > 5, "Pass", "Fail")
df
```

```
   gene  exp group Pass
1    G1  1.2     A Fail
2    G2  3.4     B Fail
3    G3  5.6     A Pass
4    G4  7.8     B Pass
5    G5  9.0     A Pass
6    G6  2.3     B Fail
7    G7  4.5     A Fail
8    G8  6.7     B Pass
9    G9  8.9     A Pass
10  G10 10.1     B Pass
```

Explanation Data Frames: A data frame is a table-like structure where each column can have a different data type (e.g., `numeric`, `character`). Accessing Columns: Use `$` to access a specific column by name. Subsetting Rows: Use logical conditions to filter rows based on specific criteria. Adding Columns: You can add new columns to a data frame using the `$` operator. In the example, a new column Pass is added based on the condition that exp is greater than 5. Exercise: Create a data frame with at least 5 columns and 10 rows. Access specific columns and rows using `$` and `[ ]`. Subset the data frame based on specific conditions (e.g., values greater than a certain threshold).

Factors

Factors are used to represent categorical data in R. They are important for statistical modeling and data analysis. They can be ordered or unordered and are useful for representing categorical variables in data frames.

```r
# Create a factor variable
df$group <- factor(df$group, levels = c("A", "B"), labels = c("Group A", "Group B"))
df$group
```

```
 [1] Group A Group B Group A Group B Group A Group B Group A Group B Group A
[10] Group B
Levels: Group A Group B
```

```r
# Check the structure of the data frame
str(df)
```

```
'data.frame':   10 obs. of  4 variables:
 $ gene : chr  "G1" "G2" "G3" "G4" ...
 $ exp  : num  1.2 3.4 5.6 7.8 9 2.3 4.5 6.7 8.9 10.1
 $ group: Factor w/ 2 levels "Group A","Group B": 1 2 1 2 1 2 1 2 1 2
 $ Pass : chr  "Fail" "Fail" "Pass" "Pass" ...
```

```r
# Convert a character vector to a factor
df$gene <- factor(df$gene)
df$gene
```

```
 [1] G1  G2  G3  G4  G5  G6  G7  G8  G9  G10
Levels: G1 G10 G2 G3 G4 G5 G6 G7 G8 G9
```

```r
# Check the levels of the factor
levels(df$gene)
```

```
 [1] "G1"  "G10" "G2"  "G3"  "G4"  "G5"  "G6"  "G7"  "G8"  "G9"
```

```r
# Convert a factor back to a character vector
df$gene <- as.character(df$gene)
df$gene
```

```
 [1] "G1"  "G2"  "G3"  "G4"  "G5"  "G6"  "G7"  "G8"  "G9"  "G10"
```

Explanation Factors: Factors are used to represent categorical data in R. They are important for statistical modeling and data analysis. Creating Factors: Use the `factor()` function to create a factor variable. You can specify the levels and labels for better readability. Converting Factors: You can convert a factor back to a character vector using `as.character()`. Exercise: Create a factor variable for the group column in the data frame. Check the levels of the factor and convert it back to a character vector.

5 Wrangling with dplyr

Explanation of dplyr Verbs The dplyr package provides a set of functions (verbs) for data manipulation. These functions are intuitive and work seamlessly with data frames and tibbles.

```r
library(dplyr)
```

```

Attaching package: 'dplyr'

The following objects are masked from 'package:stats':

    filter, lag

The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

Verb	Description	Example
`select()`	choose columns	select(df, gene, expr)
`filter()`	choose rows	filter(df, expr > 6)
`mutate()`	add / transform column	mutate(df, log2expr = log2(expr))
`arrange()`	sort rows	arrange(df, desc(expr))
`group_by()`	split into groups	group_by(df, gene)
`summarise()`	aggregate per group	summarise(df, mean(expr))

5.1 select(): Choose Columns

```r
# Select specific columns
df_selected <- select(df, gene, exp)
df_selected
```

```
   gene  exp
1    G1  1.2
2    G2  3.4
3    G3  5.6
4    G4  7.8
5    G5  9.0
6    G6  2.3
7    G7  4.5
8    G8  6.7
9    G9  8.9
10  G10 10.1
```

Description: The `select()` function is used to choose specific columns from a data frame. Example: In the example above, only the gene and exp columns are selected from the data frame.

5.2 filter(): Choose Rows

```r
# Filter rows based on a condition
df_filtered <- filter(df, exp > 5)
df_filtered
```

```
  gene  exp   group Pass
1   G3  5.6 Group A Pass
2   G4  7.8 Group B Pass
3   G5  9.0 Group A Pass
4   G8  6.7 Group B Pass
5   G9  8.9 Group A Pass
6  G10 10.1 Group B Pass
```

Description: The `filter()` function is used to subset rows based on logical conditions. Example: In the example above, only rows where exp is greater than 5 are selected. Exercise: Use `filter()` to extract rows where gene is "G1". Try filtering rows based on multiple conditions (e.g., exp $>$ 5 and group == "A").

5.3 mutate(): Add or Transform Columns

```r
# Add a new column
df_mutated <- mutate(df, Pass = ifelse(exp > 5, "Pass", "Fail"))
df_mutated
```

```
   gene  exp   group Pass
1    G1  1.2 Group A Fail
2    G2  3.4 Group B Fail
3    G3  5.6 Group A Pass
4    G4  7.8 Group B Pass
5    G5  9.0 Group A Pass
6    G6  2.3 Group B Fail
7    G7  4.5 Group A Fail
8    G8  6.7 Group B Pass
9    G9  8.9 Group A Pass
10  G10 10.1 Group B Pass
```

Description: The `mutate()` function is used to add new columns or modify existing ones. Example: In the example above, a new column Pass is added based on the condition that exp is greater than 5. Exercise: Add a new column that calculates the square of the exp column. Try using `mutate()` to create a new column that categorizes exp into "Low" (<= 5) and "High" (> 5).

5.4 arrange(): Sort Rows

```r
# Sort rows by Score in descending order
df_arranged <- arrange(df, desc(exp))
df_arranged
```

```
   gene  exp   group Pass
1   G10 10.1 Group B Pass
2    G5  9.0 Group A Pass
3    G9  8.9 Group A Pass
4    G4  7.8 Group B Pass
5    G8  6.7 Group B Pass
6    G3  5.6 Group A Pass
7    G7  4.5 Group A Fail
8    G2  3.4 Group B Fail
9    G6  2.3 Group B Fail
10   G1  1.2 Group A Fail
```

Description: The `arrange()` function is used to sort rows based on one or more columns. Example: In the example above, rows are sorted by exp in descending order. Exercise: Sort rows by gene in ascending order. Try sorting by multiple columns (e.g., first by group and then by exp).

5.5 group_by() and summarise(): Group and Aggregate

```r
library(dplyr)

df %>%
  group_by(group) %>%
  summarise(mean_expr = mean(exp),
            count     = n())
```

```
# A tibble: 2 x 3
  group   mean_expr count
  <fct>       <dbl> <int>
1 Group A      5.84     5
2 Group B      6.06     5
```

```r
# Group by Pass/Fail and calculate average expression
df_grouped <- df %>%
  mutate(Pass = ifelse(exp > 5, "Pass", "Fail")) %>%
  group_by(Pass) %>%
  summarise(mean_exp = mean(exp))
df_grouped
```

```
# A tibble: 2 x 2
  Pass  mean_exp
  <chr>    <dbl>
1 Fail      2.85
2 Pass      8.02
```

Description: The `group_by()` function is used to split the data into groups based on one or more columns. The `summarise()` function is then used to calculate summary statistics for each group. Example: In the example above, the data is grouped by gene, and the mean expression and count of rows are calculated for each group. Exercise: Group the data by group and calculate the mean expression for each group.

6 Reshaping with tidyr

The tidyr package is used for reshaping and tidying data. It provides functions like `pivot_longer()` and `pivot_wider()` to transform data between wide and long formats. #### 6.1 pivot_longer(): Wide to Long Format

```r
# Example: Convert wide data to long format
library(tidyr)
wide <- data.frame(id = 1:2, A = c(5,7), B = c(2,3))
wide
```

```
  id A B
1  1 5 2
2  2 7 3
```

```r
long <- wide %>%
  pivot_longer(cols = A:B,
               names_to  = "marker",
               values_to = "value")
long
```

```
# A tibble: 4 x 3
     id marker value
  <int> <chr>  <dbl>
1     1 A          5
2     1 B          2
3     2 A          7
4     2 B          3
```

Description: The `pivot_longer()` function converts wide-format data into long-format data by gathering multiple columns into key-value pairs. Example: In the example above, columns A and B are gathered into a single column marker, with their values stored in the value column. Exercise: Creat a wide data frame with 3 rows and 4 columns (e.g., id, X, Y, Z). Use `pivot_longer()` to convert it into long format. Rename the new columns to variable and measurement.

6.2 pivot_wider(): Long to Wide Format

```r
# Example: Convert long data back to wide format
wide_again <- long %>%
  pivot_wider(names_from = marker,
              values_from = value)
wide_again
```

```
# A tibble: 2 x 3
     id     A     B
  <int> <dbl> <dbl>
1     1     5     2
2     2     7     3
```

Description: The `pivot_wider()` function converts long-format data into wide-format data by spreading key-value pairs into multiple columns. Example: In the example above, the marker column is spread into separate columns A and B. Exercise: Take the long-format data from the previous exercise and convert it back to wide format. Try using `pivot_wider()` with a dataset that has multiple grouping variables.

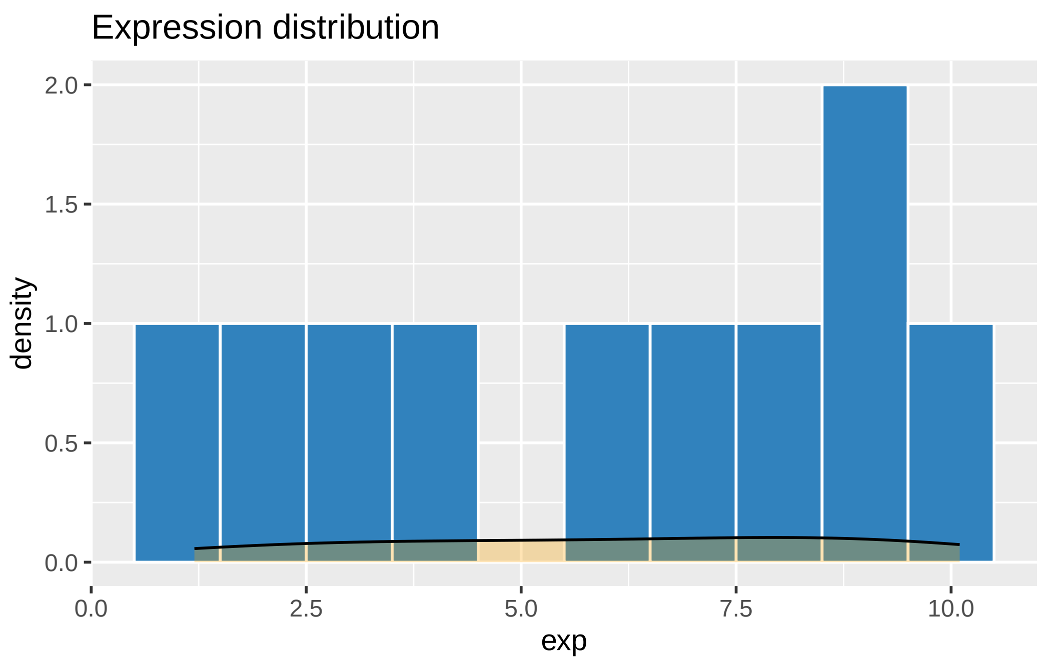
7 Visualising with ggplot2

The `ggplot2` package is a powerful tool for creating visualizations in R. It uses a layered grammar of graphics to build plots step by step.

```r
library(ggplot2)
```

7.1 Histogram + Density Plot

```r
ggplot(df, aes(exp)) +
  geom_histogram(binwidth = 1, fill = "#3182bd", colour = "white") +
  geom_density(alpha = .3, fill = "orange") +
  labs(title = "Expression distribution")
```

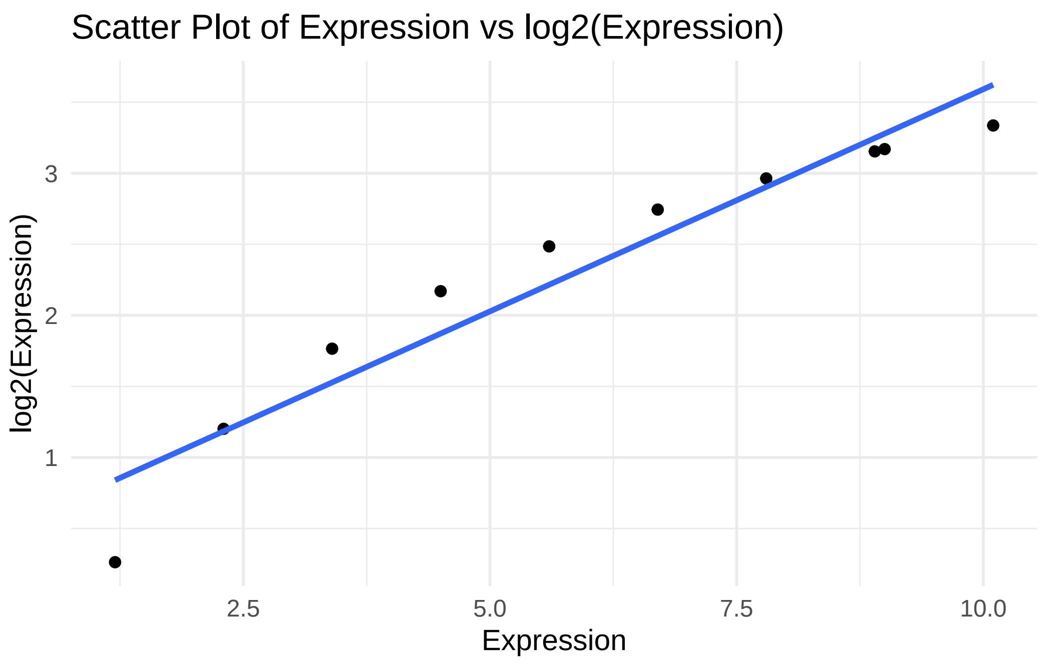


Description: - `geom_histogram()`: Creates a histogram to visualize the distribution of expression values. - `geom_density()`: Adds a density plot to show the distribution shape. - `binwidth`: Controls the width of the histogram bins. - `alpha`: Controls the transparency of the density plot. Exercise: - Create a histogram of the Age column with a binwidth of 5. - Add a density plot to the histogram. - Experiment with different fill colors and transparency levels.

7.2 Scatter Plot

```r
# Create a scatter plot
ggplot(df, aes(exp, log2(exp))) +
  geom_point() +
  geom_smooth(method = "lm", se = FALSE) +
  labs(title = "Scatter Plot of Expression vs log2(Expression)",
    x = "Expression",
    y = "log2(Expression)") +
  theme_minimal()
```

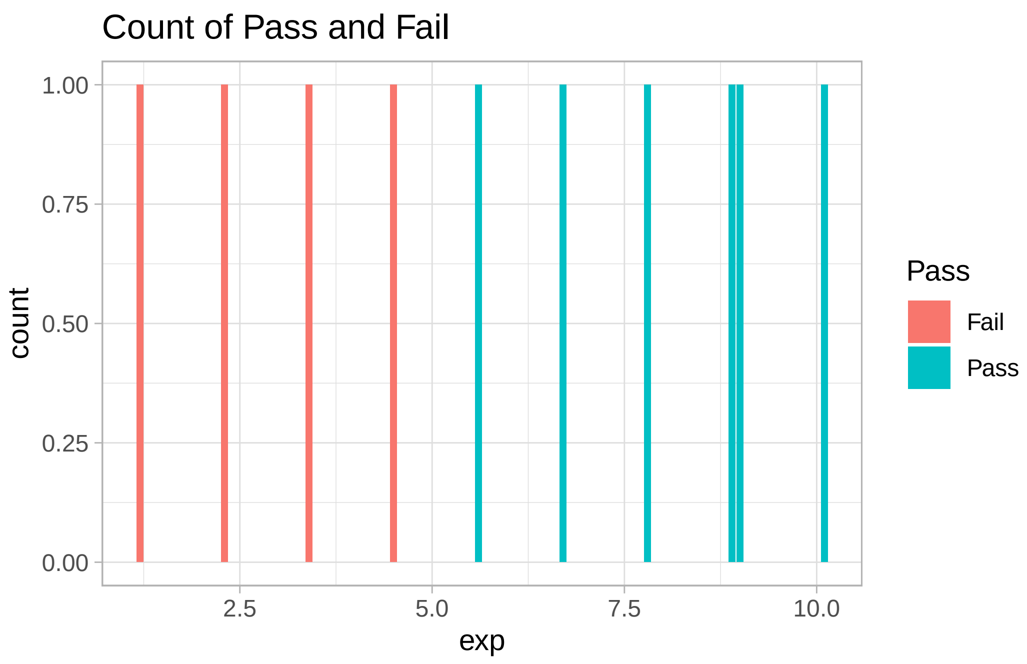
```
`geom_smooth()` using formula = 'y ~ x'
```



Description: `geom_point()`: Creates a scatter plot of expression vs log2(expression). `geom_smooth(method = "lm", se = FALSE)`: Adds a linear regression line without confidence intervals. `labs()`: Adds titles and labels to the axes. `theme_minimal()`: Applies a clean, minimal theme.

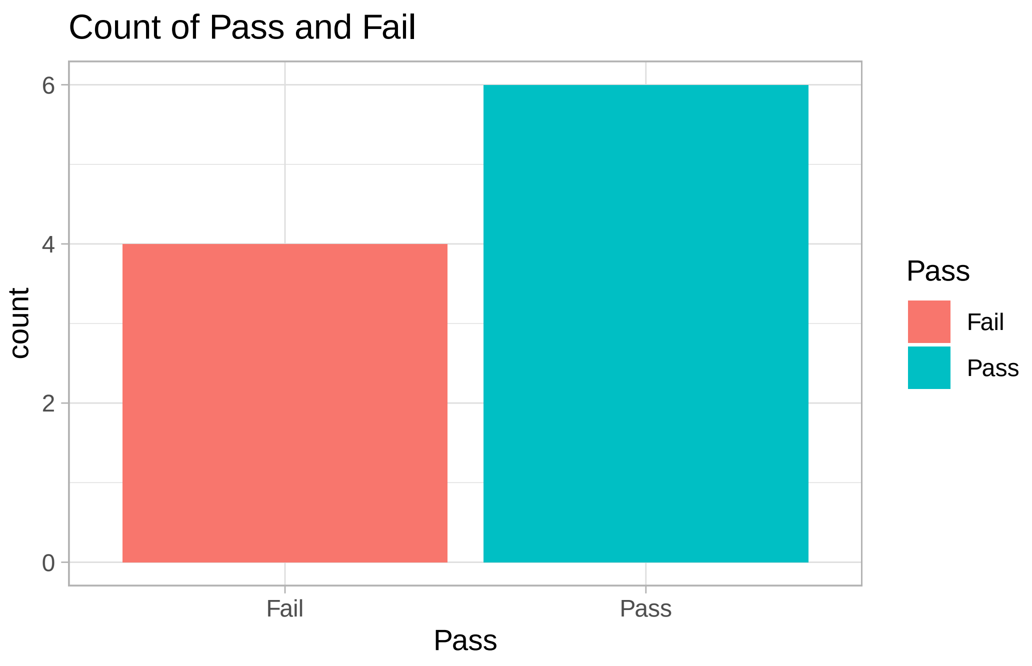
7.3 Bar Plot

```r
# Create a bar plot
ggplot(df , aes(x = exp, fill = Pass)) +
  geom_bar() +
  theme_light() +
  labs(title = "Count of Pass and Fail")
```



Description: `geom_bar()`: Creates a bar plot. `aes(fill = Pass)`: Fills the bars with colors based on the Pass column. `theme_light()`: Applies a light theme. Exercise: Create a bar plot showing the count of Pass and Fail. Experiment with different themes (e.g., theme_classic(), theme_dark()).

```
ggplot(df, aes(x = Pass, fill = Pass)) +
  geom_bar() +
  theme_light() +
  labs(title = "Count of Pass and Fail")
```



8 Full Workflow on biomed_data.csv

8.1 Load & Inspect

```r
library(readr)
biomed <- read_csv("/home/rstudio/biomed_data.csv", show_col_types = FALSE)
glimpse(biomed)      # columns & types
```

```
Rows: 200
Columns: 14
$ patient_id <chr> "P1", "P2", "P3", "P4", "P5", "P6", "P7", "P8", "P9", "P10"~
$ sex        <chr> "Female", "Female", "Female", "Female", "Male", "Female", "~
$ age        <dbl> 64, 27, 32, 43, 28, 44, 43, 31, 56, 41, 49, 59, 26, 61, 71,~
$ group      <chr> "Treated", "Treated", "Treated", "Control", "Control", "Con~
$ region     <chr> "North", "North", "North", "South", "South", "North", "Sout~
$ dose       <chr> "Low", "High", "High", "Low", "Medium", "High", "High", "Me~
$ marker_A   <dbl> 5.64, 3.61, 3.87, 3.86, 4.31, 5.17, 5.59, 4.18, 2.14, 5.95,~
$ marker_B   <dbl> 14.07, 11.44, 11.98, 13.23, 13.29, 10.17, 14.07, 8.11, 7.78~
$ marker_C   <dbl> 49.52, 46.27, 51.05, 45.76, 53.96, 50.15, 53.02, 45.03, 48.~
$ marker_D   <dbl> 1.23, 1.81, 1.91, 1.12, 0.99, 1.24, 1.54, 1.31, 0.79, 1.04,~
$ marker_E   <dbl> 11.38, 11.62, 10.65, 10.73, 9.75, 8.69, 10.27, 10.29, 9.00,~
$ expression <dbl> 8.66, 12.54, 11.99, 11.27, 8.67, 10.80, 8.90, 8.97, 6.92, 1~
$ heart_rate <dbl> 69, 88, 84, 64, 91, 100, 64, 85, 92, 91, 99, 99, 94, 94, 63~
$ RBC_count  <dbl> 4.47, 4.38, 3.99, 4.02, 5.32, 4.45, 4.73, 4.74, 4.95, 4.41,~
```

```r
summary(biomed)      # summary stats & NAs
```

```
  patient_id            sex                 age            group          
 Length:200         Length:200         Min.   :25.00   Length:200        
 Class :character   Class :character   1st Qu.:38.00   Class :character  
 Mode  :character   Mode  :character   Median :49.00   Mode  :character  
                                       Mean   :50.09                     
                                       3rd Qu.:61.25                     
                                       Max.   :75.00                     
                                                                         
    region              dose              marker_A        marker_B    
 Length:200         Length:200         Min.   :2.140   Min.   : 6.72  
 Class :character   Class :character   1st Qu.:4.300   1st Qu.:11.10  
 Mode  :character   Mode  :character   Median :4.900   Median :12.58  
                                       Mean   :4.896   Mean   :12.43  
                                       3rd Qu.:5.525   3rd Qu.:13.89  
                                       Max.   :7.350   Max.   :18.09  
                                       NA's   :5                      
    marker_C        marker_D          marker_E        expression    
 Min.   :35.35   Min.   :0.1400   Min.   : 5.850   Min.   : 4.000  
 1st Qu.:46.63   1st Qu.:0.8875   1st Qu.: 9.265   1st Qu.: 7.280  
 Median :49.93   Median :1.1200   Median :10.325   Median : 8.680  
 Mean   :49.89   Mean   :1.1838   Mean   :10.183   Mean   : 8.876  
 3rd Qu.:52.98   3rd Qu.:1.3550   3rd Qu.:11.110   3rd Qu.:10.340  
 Max.   :62.26   Max.   :2.9400   Max.   :15.040   Max.   :16.640  
 NA's   :5                                         NA's   :5       
   heart_rate        RBC_count    
 Min.   : 57.00   Min.   :3.550  
 1st Qu.: 67.00   1st Qu.:4.265  
 Median : 77.00   Median :4.550  
 Mean   : 78.89   Mean   :4.554  
 3rd Qu.: 91.00   3rd Qu.:4.860  
 Max.   :103.00   Max.   :5.490  
                  NA's   :5      
```

Explanation - `read_csv()`: Reads a CSV file into a data frame. - `glimpse()`: Provides a quick overview of the data frame, including column names and types. - `summary()`: Provides summary statistics for each column, including mean, median, and number of missing values (NAs). - show_col_types = FALSE: Suppresses the display of column types in the output.

- Exercise: - Load the `biomed_data.csv` file and inspect its structure. - Check for missing values in the dataset. - Summarize the data to understand its distribution and key statistics. - Identify any potential outliers or anomalies in the data.

8.2 Key dplyr Demonstrations

a) Select & Filter

```r
# Keep only patient_id, age, group, expression
biomed %>%
  select(patient_id, age, group, expression) %>%
  head(5)
```

```
# A tibble: 5 x 4
  patient_id   age group   expression
  <chr>      <dbl> <chr>        <dbl>
1 P1            64 Treated       8.66
2 P2            27 Treated      12.5
3 P3            32 Treated      12.0
4 P4            43 Control      11.3
5 P5            28 Control       8.67
```

```r
# Keep only patient_id, age, group, expression
biomed_filtered <- biomed %>%
  select(patient_id, age, group, expression) %>%
  filter(age > 30 & group == "Treated")
biomed_filtered
```

```
# A tibble: 85 x 4
   patient_id   age group   expression
   <chr>      <dbl> <chr>        <dbl>
 1 P1            64 Treated       8.66
 2 P3            32 Treated      12.0
 3 P8            31 Treated       8.97
 4 P11           49 Treated       7.88
 5 P15           71 Treated       9.45
 6 P16           71 Treated       6.17
 7 P17           54 Treated      12.7
 8 P18           34 Treated      11.2
 9 P20           73 Treated       7.29
10 P21           59 Treated       9.22
# i 75 more rows
```

Description: - `select()`: Keeps only the specified columns (patient_id, age, group, expression). - `filter()`: Filters rows where age > 30 and group is “Treated”. Exercise: Select only the patient_id, group, and expression columns. Filter rows where expression is greater than 50 and group is “Control”.

```
# Patients older than 60 in Treated group
biomed %>%
  filter(age > 60, group == "Treated") %>%
  select(patient_id, age, group)
```

```
# A tibble: 29 x 3
   patient_id   age group
   <chr>      <dbl> <chr>
 1 P1            64 Treated
 2 P15           71 Treated
 3 P16           71 Treated
 4 P20           73 Treated
 5 P27           61 Treated
 6 P30           71 Treated
 7 P40           72 Treated
 8 P61           66 Treated
 9 P62           61 Treated
10 P64           66 Treated
# i 19 more rows
```

Description: - `filter()`: Filters rows where age is greater than 60 and group is “Treated”. - `select()`: Keeps only the patient_id, age, and group columns. Exercise: - Filter patients with expression > 10 and group “Control”. - Select only the patient_id, age, and expression columns.

b) Mutate & Arrange

```
# Add a new column for age category and sort by expression
biomed_mutated <- biomed %>%
  mutate(age_category = ifelse(age > 50, "Senior", "Adult")) %>%
  arrange(desc(expression))
biomed_mutated
```

```
# A tibble: 200 x 15
   patient_id sex     age group region dose   marker_A marker_B marker_C marker_D
   <chr>      <chr> <dbl> <chr> <chr>  <chr>     <dbl>    <dbl>    <dbl>    <dbl>
```

```
 1 P54         Fema~    59 Trea~ North  High       3.31     12.8       NA        2.15
 2 P127        Fema~    29 Trea~ East   High       5.19     15.1       51.3      2.05
 3 P30         Fema~    71 Trea~ South  High       4.57      9.95      43.8      1.28
 4 P168        Fema~    70 Trea~ South  Low        2.34     16.4       54.5      0.14
 5 P91         Male     37 Trea~ North  High       6.26     13.4       53.4      1.82
 6 P140        Fema~    48 Trea~ North  High       5.25     14.1       44.1      2.64
 7 P10         Fema~    41 Cont~ North  High       5.95     12.5       51.7      1.04
 8 P165        Fema~    48 Trea~ North  High       5.14     12.4       49.3      1.96
 9 P79         Fema~    68 Trea~ East   High       4.62     12.5       53.6      1.99
10 P102        Male     62 Trea~ West   High       4.35     11.0       48.9      2.94
# i 190 more rows
# i 5 more variables: marker_E <dbl>, expression <dbl>, heart_rate <dbl>,
#   RBC_count <dbl>, age_category <chr>
```

Description: - `mutate()`: Adds a new column (age_category) that categorizes age into "Senior" (>50) and "Adult" (<=50). - `arrange()`: Sorts the data frame by expression in descending order.

Exercise: Create a new column that categorizes expression into "High" (>7) and "Low" (<=7). Sort the data by age in ascending order.

c) Group & Summarise

```r
# Mean expression & count per group & dose
biomed %>%
  group_by(group, dose) %>%
  summarise(mean_expr = mean(expression, na.rm = TRUE),
            sd_expr   = sd(expression, na.rm = TRUE),
            n         = n())
```

```
`summarise()` has grouped output by 'group'. You can override using the
`.groups` argument.
```

```
# A tibble: 6 x 5
# Groups:   group [2]
  group   dose   mean_expr sd_expr     n
  <chr>   <chr>      <dbl>   <dbl> <int>
1 Control High        8.42    2.01    37
2 Control Low         8.63    1.79    34
3 Control Medium      7.89    1.88    34
4 Treated High       11.8     1.84    34
5 Treated Low         8.41    1.83    32
6 Treated Medium      7.90    1.77    29
```

Description: - `group_by()`: Groups the data by group and dose. - `summarise()`: Calculates the mean expression, standard deviation, and count of rows for each group and dose. Exercise: - Group the data by group and dose, and calculate the mean expression and standard deviation for each group.

```r
# Group by group and calculate mean expression
biomed_summary <- biomed %>%
  group_by(group) %>%
  summarise(mean_expression = mean(expression, na.rm = TRUE),
            count = n())
biomed_summary
```

```
# A tibble: 2 x 3
  group   mean_expression count
  <chr>             <dbl> <int>
1 Control            8.31   105
2 Treated            9.50    95
```

Description: `group_by()`: Groups the data by the group column. `summarise()`: Calculates the mean expression and the count of rows for each group. Exercise: Group the data by age_category and calculate the median expression for each category. Add a column to count the number of patients in each group.

```r
# Frequency table of region
biomed %>%
  count(region) %>%
  arrange(desc(n))
```

```
# A tibble: 4 x 2
  region     n
  <chr>  <int>
1 East      59
2 North     51
3 South     50
4 West      40
```

d) Full Pipeline Example

```
# Combine all steps into a single pipeline    <!-- filter(age > 30 & group == "Treatment") %
biomed_pipeline <- biomed %>%
  select(patient_id, age, group, expression) %>%

  mutate(age_category = ifelse(age > 50, "Senior", "Young")) %>%
  group_by(age_category) %>%
  summarise(mean_expression = mean(expression, na.rm = TRUE),
            count = n()) %>%
  arrange(desc(mean_expression))
biomed_pipeline
```

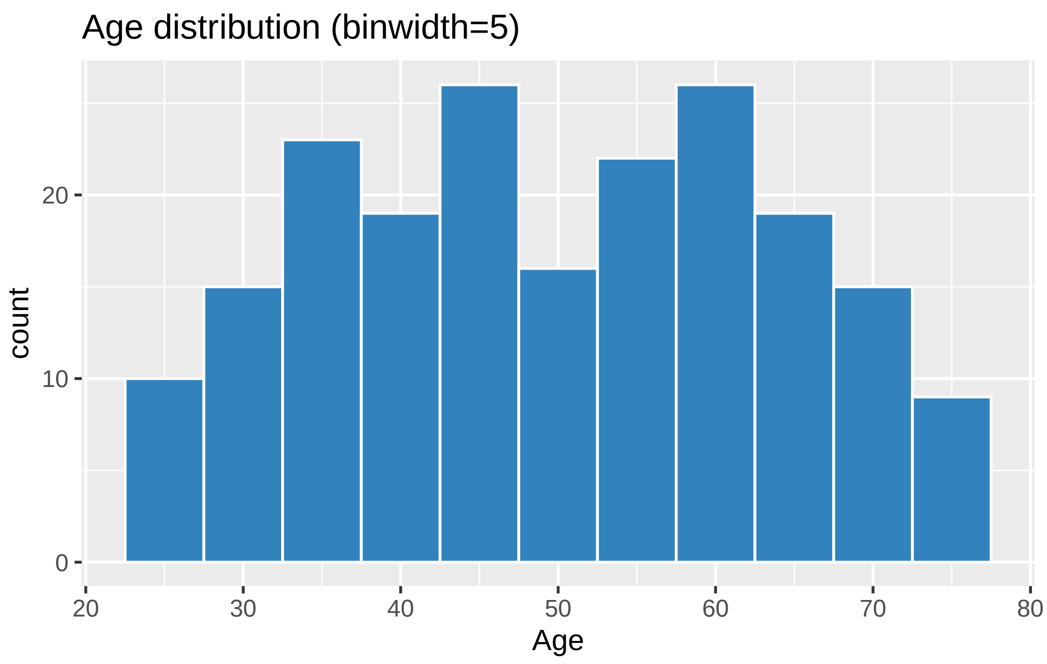
```
# A tibble: 2 x 3
  age_category mean_expression count
  <chr>                  <dbl> <int>
1 Young                   9.37   101
2 Senior                  8.36    99
```

Description: This pipeline combines `select()`, `filter()`, `mutate()`, `group_by()`, `summarise()`, and `arrange()` into a single workflow. Exercise: Modify the pipeline to include only patients with expression > 50. Add a step to calculate the standard deviation of expression for each age_category.

8.3 Extensive ggplot2 Gallery

Histogram of Age

```
ggplot(biomed, aes(age)) +
  geom_histogram(binwidth = 5, fill = "#3182bd", colour = "white") +
  labs(title = "Age distribution (binwidth=5)", x = "Age")
```

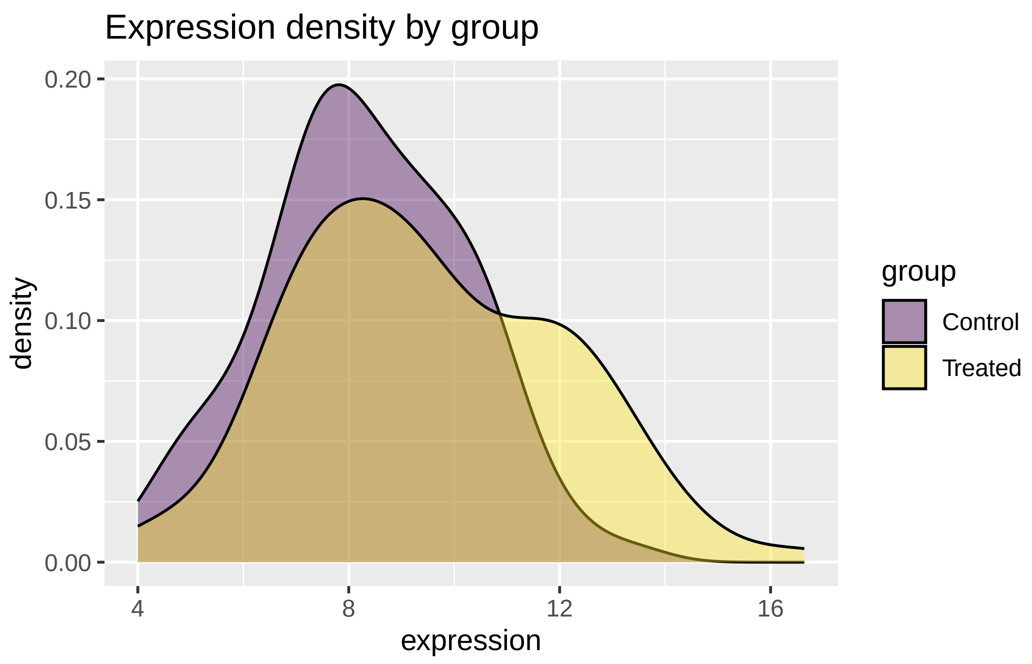


Explanation - `geom_histogram()`: Creates a histogram to visualize the distribution of age values. - `binwidth`: Controls the width of the histogram bins. - `labs()`: Adds titles and labels to the axes. - Exercise: - Create a histogram of expression with a binwidth of 10. - Add a density plot to the histogram. - Experiment with different fill colors and transparency levels.

Density of Expression by Group

```r
ggplot(biomed, aes(expression, fill = group)) +
  geom_density(alpha = .4) +
  scale_fill_viridis_d() +
  labs(title = "Expression density by group")
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_density()`).
```

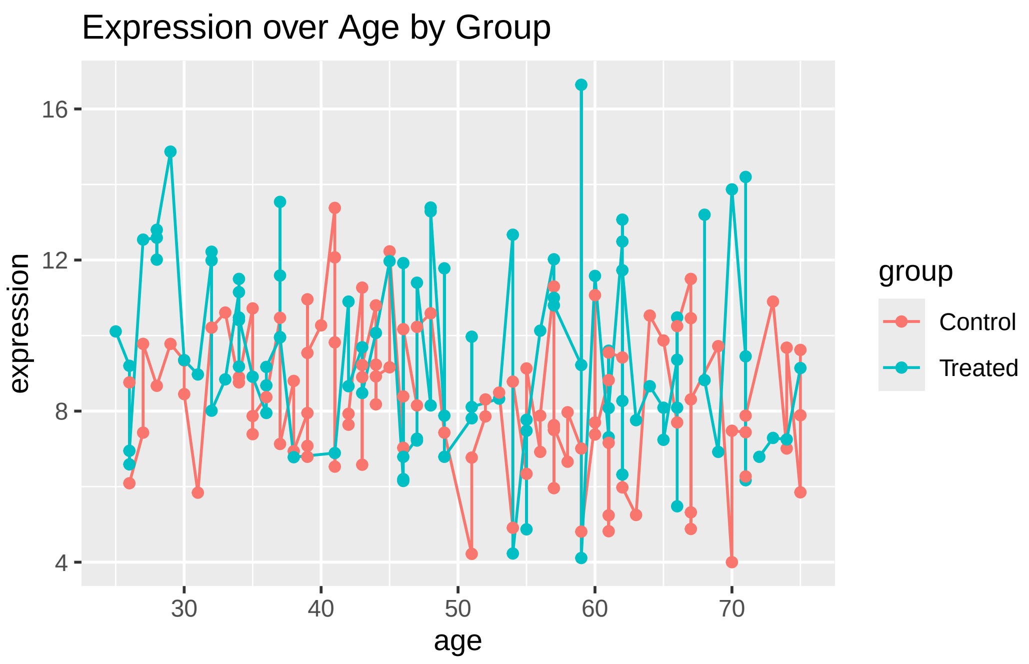


Explanation - `geom_density()`: Creates a density plot to visualize the distribution of expression values by group. - `alpha`: Controls the transparency of the density plot. - `scale_fill_viridis_d()`: Applies a color palette for better visibility. - Exercise: - Create a density plot of heart_rate by region. - Experiment with different fill colors and transparency levels.

a) Line Plot

```r
# Line plot showing trends over time
ggplot(biomed, aes(x = age, y = expression, group = group, colour = group)) +
  geom_line() +
  geom_point() +
  labs(title = "Expression over Age by Group")
```

```
Warning: Removed 5 rows containing missing values or values outside the scale range
(`geom_point()`).
```

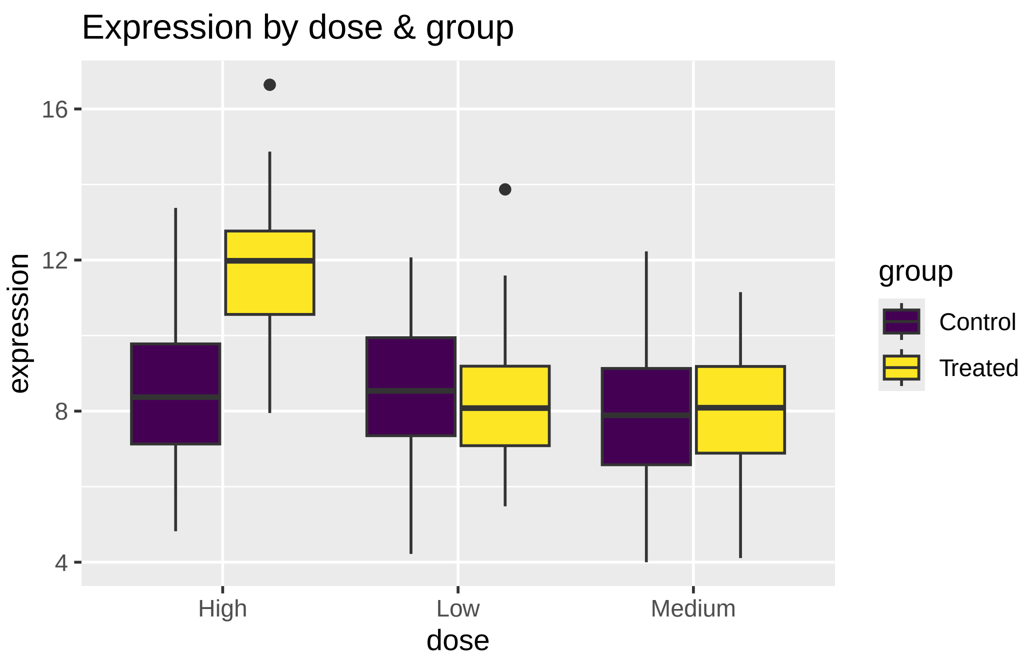


xplanation - **`geom_line()`**: Creates a line plot to visualize trends over time (or age). - **`geom_point()`**: Adds points to the line plot for better visibility. - **`group`**: Groups the data by the group variable. - **`colour`**: Colors the lines and points based on the group variable. - Exercise: - Create a line plot of heart_rate over age by region.

b) Boxplot: Expression by Dose & Group

```
ggplot(biomed, aes(dose, expression, fill = group)) +
  geom_boxplot(position = position_dodge(width = .8)) +
  scale_fill_viridis_d() +
  labs(title = "Expression by dose & group")
```

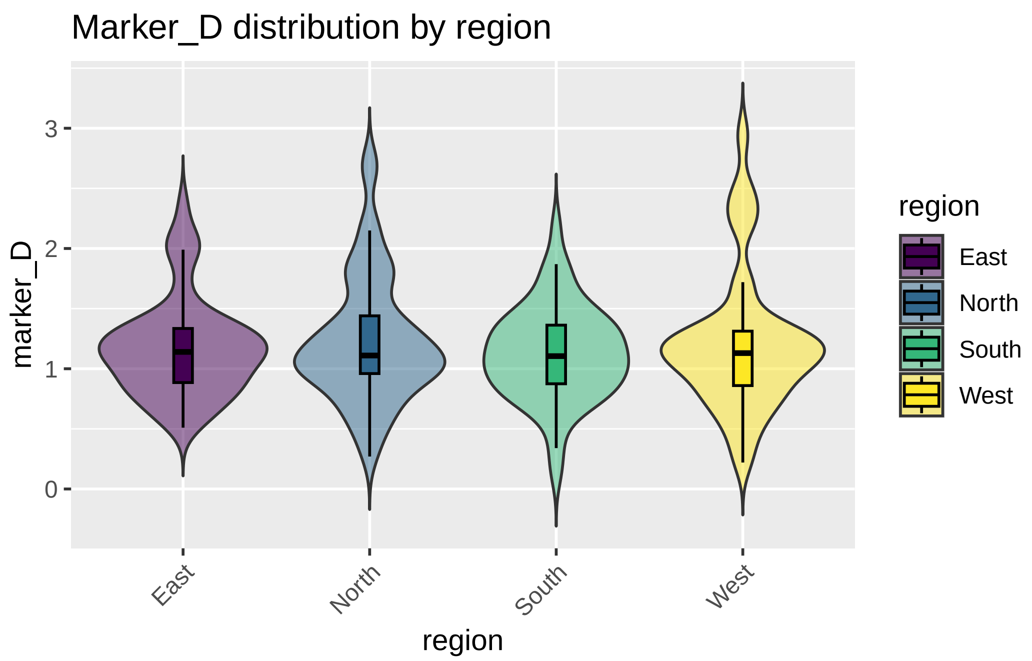
```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



Explanation - `geom_boxplot()`: Creates a boxplot to visualize the distribution of expression values by dose and group. - `position_dodge()`: Adjusts the position of the boxplots to avoid overlap. - `scale_fill_viridis_d()`: Applies a color palette for better visibility. Exercise: - Create a boxplot for marker_A by region. - Add jittered points to the boxplot to show individual data points.

c) Violin: Marker_D by Region

```
ggplot(biomed, aes(region, marker_D, fill = region)) +
  geom_violin(trim = FALSE, alpha = .5) +
  geom_boxplot(width = .1, colour = "black", outlier.shape = NA) +
  scale_fill_viridis_d() +
  labs(title = "Marker_D distribution by region") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```



Explanation - `geom_violin()`: Creates a violin plot to visualize the distribution of marker_D values by region. - `geom_boxplot()`: Adds a boxplot inside the violin plot for better summary statistics. - trim = FALSE: Ensures the violin plot is not trimmed at the tails. - `theme(axis.text.x = element_text(angle = 45, hjust = 1))`: Rotates x-axis labels for better readability. Exercise: - Create a violin plot for marker_E by group. - Add a boxplot inside the violin plot to show summary statistics.

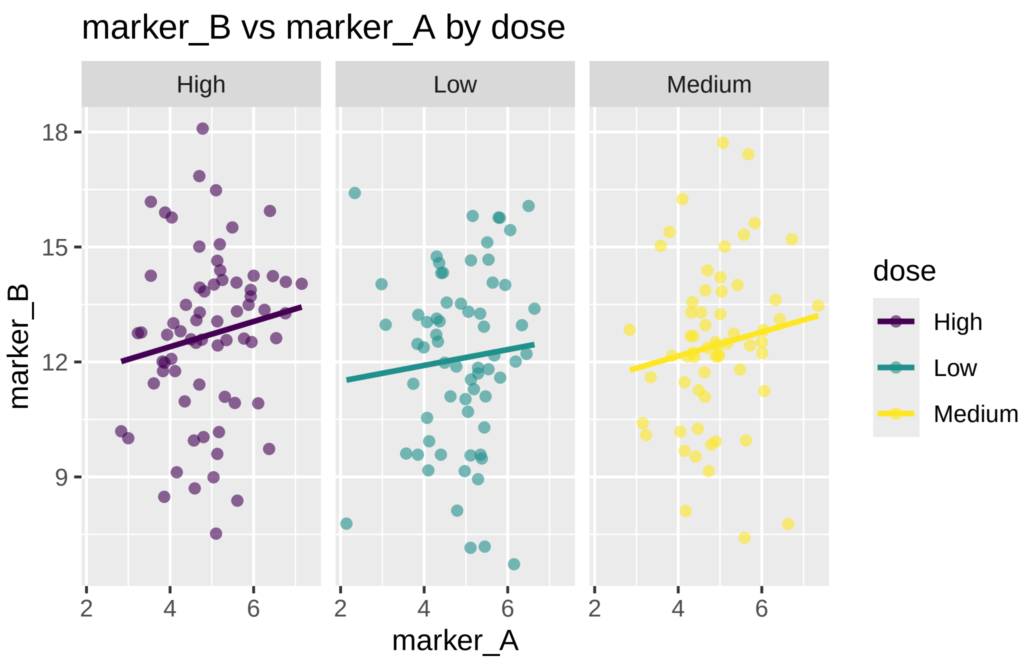
d) Scatter: marker_B vs marker_A, Faceted by Dose

```r
ggplot(biomed, aes(marker_A, marker_B, colour = dose)) +
  geom_point(alpha = .6) +
  geom_smooth(method = "lm", se = FALSE) +
  facet_wrap(~ dose) +
  scale_colour_viridis_d() +
  labs(title = "marker_B vs marker_A by dose")
```

```
`geom_smooth()` using formula = 'y ~ x'
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_smooth()`).
```

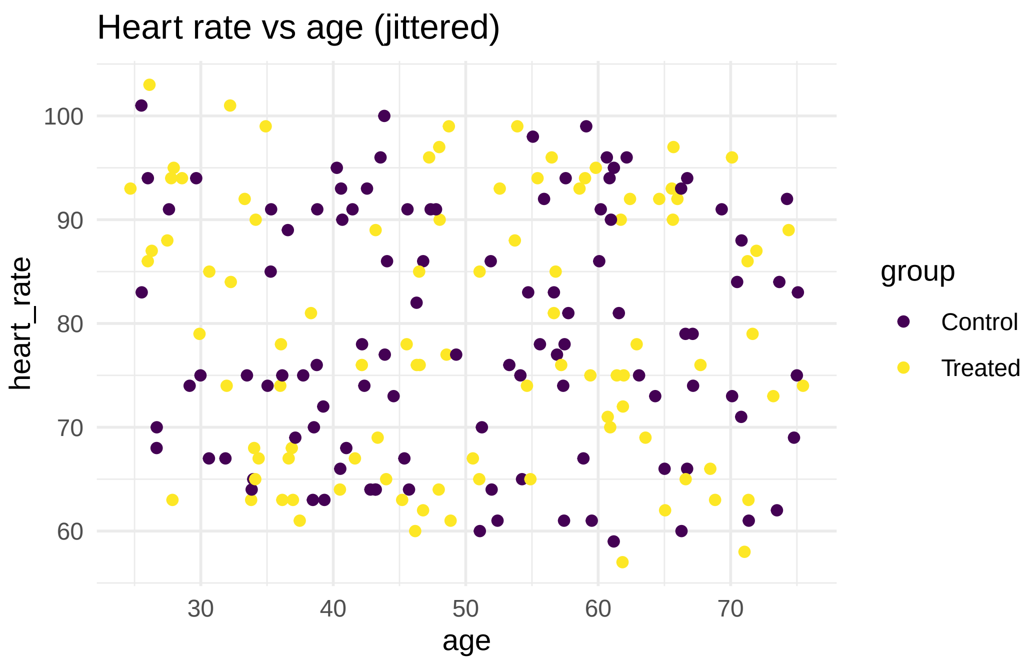
```
Warning: Removed 5 rows containing missing values or values outside the scale range
(`geom_point()`).
```



Explanation - `geom_point()`: Creates a scatter plot of marker_B vs marker_A. - `geom_smooth(method = "lm", se = FALSE)`: Adds a linear regression line without confidence intervals. - `facet_wrap(~ dose)`: Creates separate panels for each dose level. - `scale_colour_viridis_d()`: Applies a color palette for better visibility. Exercise: - Create a scatter plot of heart_rate vs age, colored by group. - Add a linear regression line to the scatter plot.

e) Jittered Scatter: Heart Rate vs Age

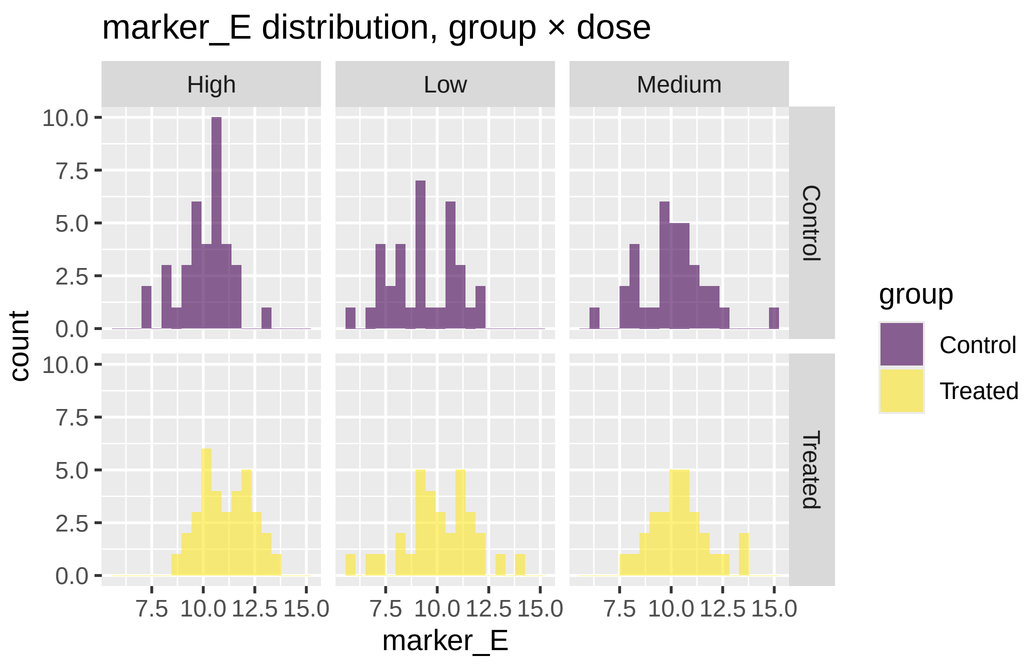
```
ggplot(biomed, aes(age, heart_rate, colour = group)) +
  geom_jitter(width = .5, height = 0) +
  scale_colour_viridis_d() +
  labs(title = "Heart rate vs age (jittered)") +
  theme_minimal()
```



Explanation - `geom_jitter()`: Creates a scatter plot with jitter to avoid overplotting. - `width and height`: Control the amount of jitter in the x and y directions. - `theme_minimal()`: Applies a clean, minimal theme. Exercise: - Create a jittered scatter plot of marker_C vs age, colored by region.

f) Facet Grid: marker_E by Group & Dose

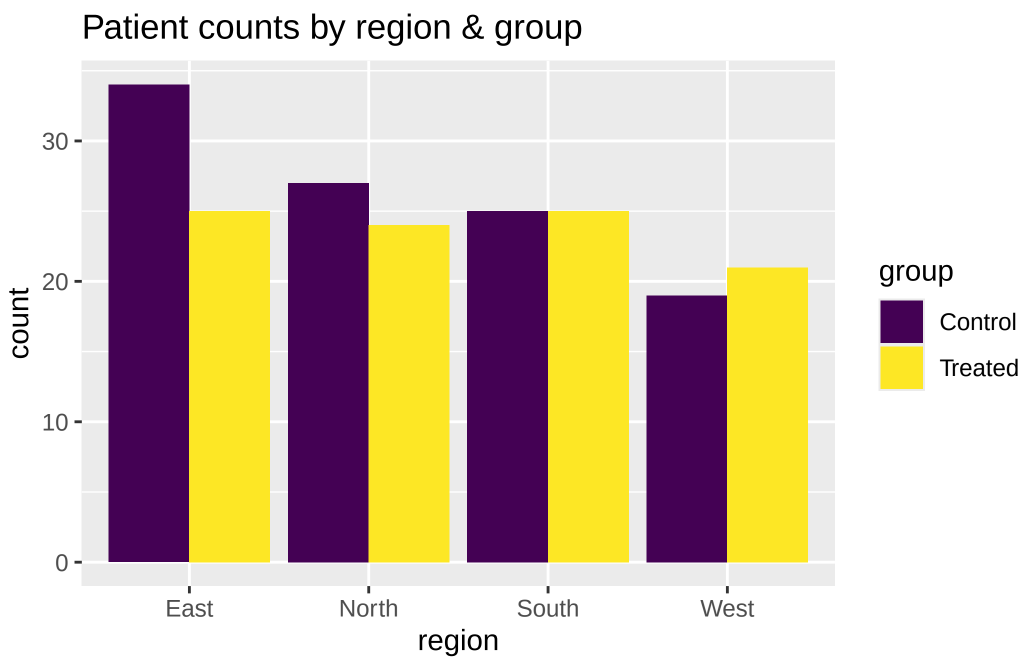
```
ggplot(biomed, aes(marker_E, fill = group)) +
  geom_histogram(bins = 20, alpha = .6) +
  facet_grid(group ~ dose) +
  scale_fill_viridis_d() +
  labs(title = "marker_E distribution, group × dose")
```



Explanation - `facet_grid(group ~ dose)`: Creates a grid of plots based on group and dose. - `geom_histogram(bins = 20, alpha = .6)`: Creates histograms for marker_E with 20 bins. - `scale_fill_viridis_d()`: Applies a color palette for better visibility. Exercise: - Create a facet grid of marker_A by region and group.

g) Bar Chart: Count per Region & Group

```
ggplot(biomed, aes(region, fill = group)) +
  geom_bar(position = "dodge") +
  scale_fill_viridis_d() +
  labs(title = "Patient counts by region & group")
```



Explanation - `geom_bar(position = "dodge")`: Creates a bar chart showing counts of patients by region and group. - `scale_fill_viridis_d()`: Applies a color palette for better visibility. - `labs(title = "Patient counts by region & group")`: Adds a title to the plot. Exercise: - Create a bar chart showing counts of patients by age category and group. h) Pairwise Scatterplot Matrix

```
library(GGally)
```

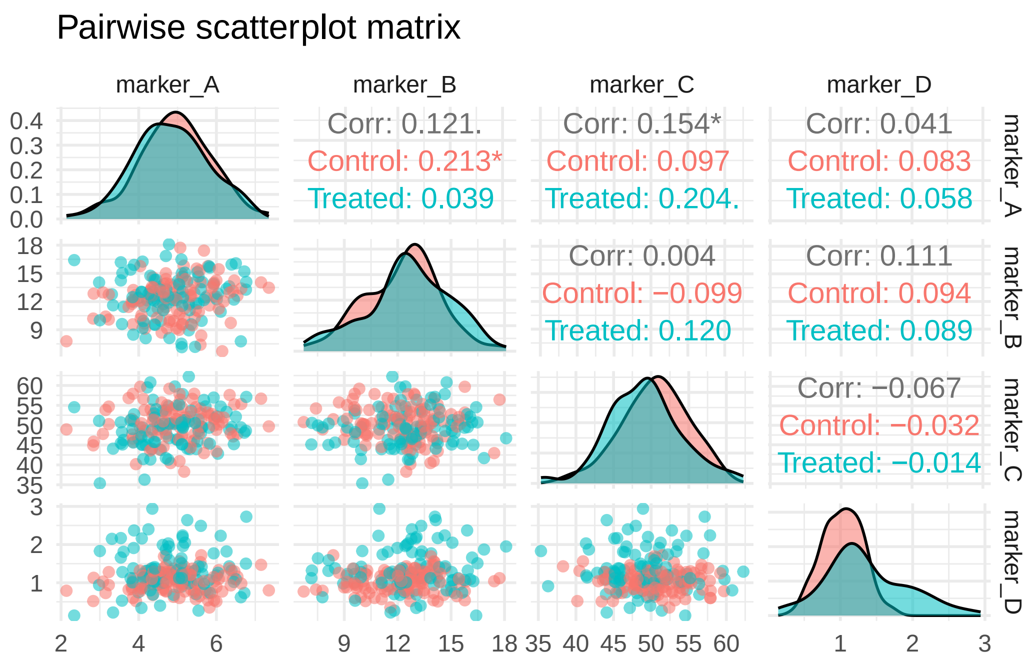
```
Registered S3 method overwritten by 'GGally':
  method from
  +.gg   ggplot2
```

```
# Create a pairwise scatterplot matrix
ggpairs(biomed, columns = c("marker_A", "marker_B", "marker_C", "marker_D"),
        aes(colour = group, alpha = 0.5)) +
  theme_minimal() +
  labs(title = "Pairwise scatterplot matrix")
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_density()`).
```

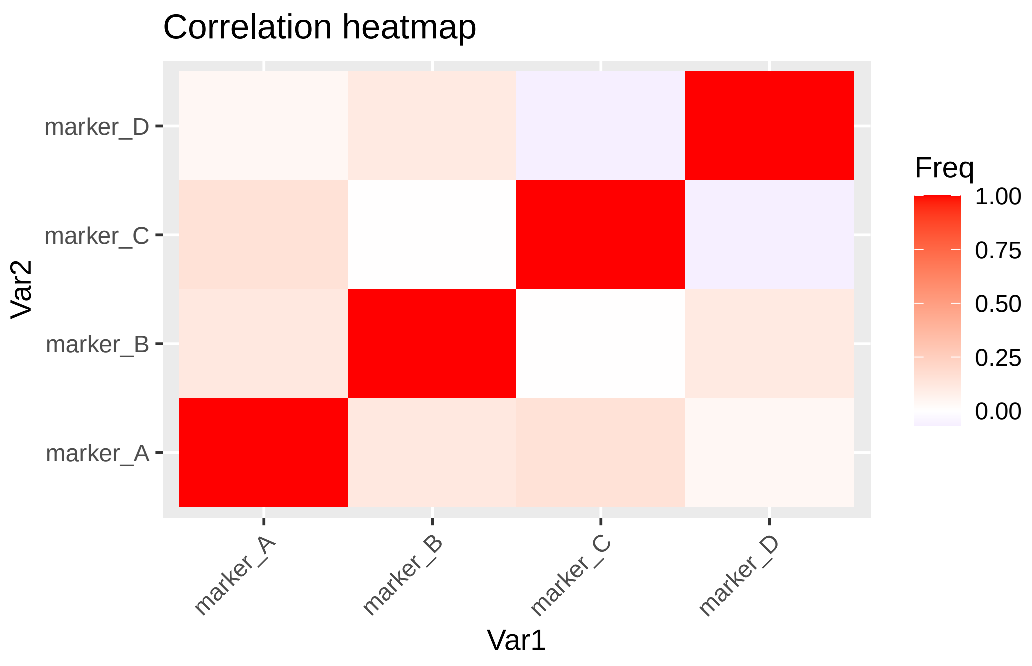
```
Warning in ggally_statistic(data = data, mapping = mapping, na.rm = na.rm, :
Removed 5 rows containing missing values
```

```
Warning in ggally_statistic(data = data, mapping = mapping, na.rm = na.rm, :
Removed 10 rows containing missing values

Warning in ggally_statistic(data = data, mapping = mapping, na.rm = na.rm, :
Removed 5 rows containing missing values

Warning: Removed 5 rows containing missing values or values outside the scale range
(`geom_point()`).

Warning in ggally_statistic(data = data, mapping = mapping, na.rm = na.rm, :
Removed 5 rows containing missing values

Warning: Removed 10 rows containing missing values or values outside the scale range
(`geom_point()`).

Warning: Removed 5 rows containing missing values or values outside the scale range
(`geom_point()`).

Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_density()`).

Warning in ggally_statistic(data = data, mapping = mapping, na.rm = na.rm, :
Removed 5 rows containing missing values

Warning: Removed 5 rows containing missing values or values outside the scale range
(`geom_point()`).
Removed 5 rows containing missing values or values outside the scale range
(`geom_point()`).
```



Explanation - `ggpairs()`: Creates a matrix of scatterplots for selected columns. - `aes(colour = group, alpha = 0.5)`: Colors the points based on the group and sets transparency. - `theme_minimal()`: Applies a clean, minimal theme. - `labs(title = "Pairwise scatterplot matrix")`: Adds a title to the plot. Exercise: - Create a pairwise scatterplot matrix for heart_rate, RBC_count, and expression. i) Correlation Matrix

```
# Compute correlation matrix
cor_matrix <- cor(biomed[, c("marker_A", "marker_B", "marker_C", "marker_D")], use = "pairwi
# Convert to long form
library(tidyr)
# Convert correlation matrix to long format without using rownames_to_column
Long <- as.data.frame(as.table(cor_matrix))
# Plot heatmap
ggplot(Long, aes(Var1, Var2, fill = Freq)) +
  geom_tile() +
  scale_fill_gradient2(low = "blue", mid = "white", high = "red",
                       midpoint = 0) +
  labs(title = "Correlation heatmap") +
  theme(axis.text.x = element_text(angle=45, hjust=1))
```

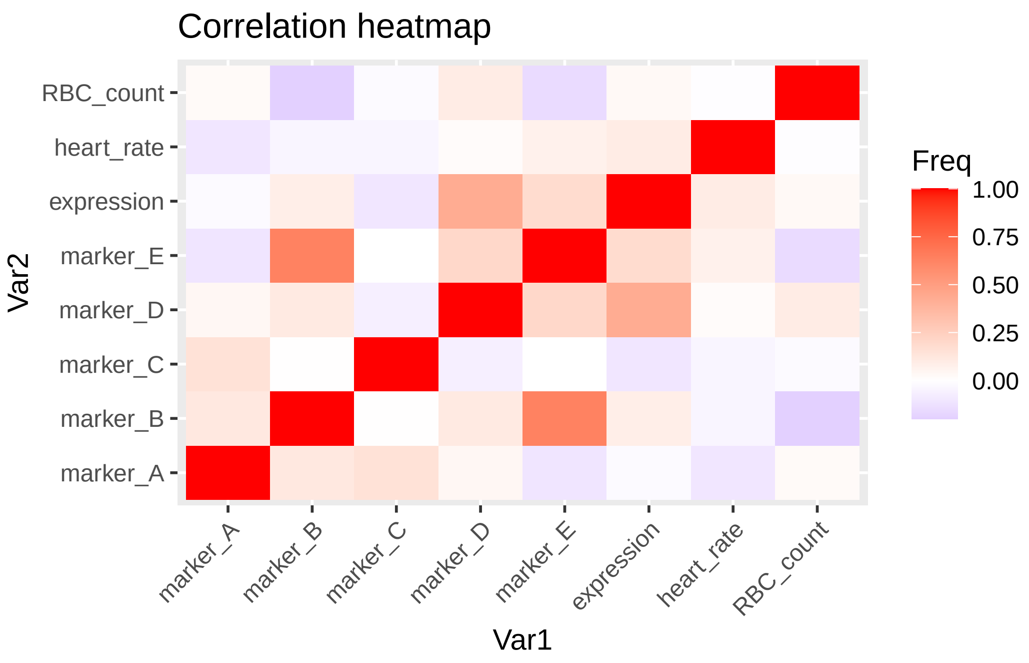


Explanation - `cor()`: Computes the correlation matrix for selected columns. - `as.table()`: Converts the correlation matrix to a table format. - `ggplot()`: Creates a heatmap of the correlation matrix. - `geom_tile()`: Creates a heatmap of the correlation matrix. - `scale_fill_gradient2()`: Sets the color gradient for the heatmap. - `labs(title = "Correlation heatmap")`: Adds a title to the plot. - `theme(axis.text.x = element_text(angle=45, hjust=1))`: Rotates x-axis labels for better readability. Exercise: - Create a correlation matrix for heart_rate, RBC_count, and expression. - Plot the correlation matrix as a heatmap.

j) Correlation Heatmap of Numeric Variables

```
# Compute correlation matrix
nums <- biomed %>%
  select(marker_A, marker_B, marker_C, marker_D,
         marker_E, expression, heart_rate, RBC_count) %>%
  cor(use = "pairwise.complete.obs")

# Convert to long form
library(tidyr)
# corr_long <- as.data.frame(nums) %>%
#   rownames_to_column("var1") %>%
#   pivot_longer(-var1, names_to="var2", values_to="corr")
#
# # Plot heatmap
```

```r
# ggplot(corr_long, aes(var1, var2, fill = corr)) +
#   geom_tile() +
#   scale_fill_gradient2(low = "blue", mid = "white", high = "red",
#                        midpoint = 0) +
#   labs(title = "Correlation heatmap") +
#   theme(axis.text.x = element_text(angle=45, hjust=1))
library(tidyr)
Long <- as.data.frame(as.table(nums))
# Plot heatmap
ggplot(Long, aes(Var1, Var2, fill = Freq)) +
  geom_tile() +
  scale_fill_gradient2(low = "blue", mid = "white", high = "red",
                       midpoint = 0) +
  labs(title = "Correlation heatmap") +
  theme(axis.text.x = element_text(angle=45, hjust=1))
```



Explanation - `cor()`: Computes the correlation matrix for selected columns. - `rownames_to_column()`: Converts row names to a column for easier plotting. - `pivot_longer()`: Reshapes the correlation matrix into long format. - `geom_tile()`: Creates a heatmap of the correlation matrix. - `scale_fill_gradient2()`: Sets the color gradient for the heatmap. - `labs(title = "Correlation heatmap")`: Adds a title to the plot. Exercise: - Create a correlation matrix for heart_rate, RBC_count, and expression. - Plot the correlation matrix as a heatmap.

References & Resources

Below is a curated list of books, websites, cheat-sheets and tutorials to deepen your understanding of R, the tidyverse, and biomedical data analysis.

Books

- **R for Data Science**
  Wickham, H. & Grolemund, G. (2016). *R for Data Science.* O'Reilly Media.
  Online: https://r4ds.had.co.nz/ :contentReferenceoaicite:0
- **ggplot2: Elegant Graphics for Data Analysis**
  Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis.* Springer.
  Online reference: https://ggplot2.tidyverse.org/ :contentReferenceoaicite:1
- **Advanced R**
  Wickham, H. (2019). *Advanced R.* Chapman & Hall/CRC.
  Online: https://adv-r.hadley.nz/ :contentReferenceoaicite:2

Official Documentation

- **tidyverse** ("meta-package" including dplyr, tidyr, ggplot2, readr, etc.)
  https://www.tidyverse.org/ :contentReferenceoaicite:3
- **dplyr reference**
  https://dplyr.tidyverse.org/reference/ :contentReferenceoaicite:4
- **tidyr reference**
  https://tidyr.tidyverse.org/reference/ :contentReferenceoaicite:5
- **ggplot2 reference**
  https://ggplot2.tidyverse.org/reference/ :contentReferenceoaicite:6
- **readr reference**
  https://readr.tidyverse.org/reference/ :contentReferenceoaicite:7

Cheat-Sheets & Quick Guides

- **RStudio Cheat-Sheets**
  Download PDF versions for dplyr, tidyr, ggplot2, and more:
  https://www.rstudio.com/resources/cheatsheets/ :contentReferenceoaicite:8
- **Tidyverse Style Guide**
  Conventions for writing tidyverse-style R code:
  https://style.tidyverse.org/ :contentReferenceoaicite:9

Online Tutorials & Courses

- **Swirl**: Interactive R lessons in the R console
  https://swirlstats.com/ :contentReferenceoaicite:10
- **Coursera: R Programming** (Johns Hopkins University)
  https://www.coursera.org/learn/r-programming :contentReferenceoaicite:11
- **Datacamp: Introduction to R**
  https://www.datacamp.com/courses/free-introduction-to-r :contentReferenceoaicite:12

Biomedical - Specific Resources

- **Bioconductor**: R packages for bioinformatics
  https://www.bioconductor.org/ :contentReferenceoaicite:13
- **Bioconductor Workflow Guides**
  E.g. RNA-seq, single-cell analyses:
  https://www.bioconductor.org/packages/release/BiocViews.html#___Workflow :contentReferenceoaicite:14

Community & Help

- **RStudio Community**
  https://community.rstudio.com/ :contentReferenceoaicite:15
- **Stack Overflow (R tag)**
  https://stackoverflow.com/questions/tagged/r :contentReferenceoaicite:16
- **R-Bloggers** (aggregated R tutorials and news)
  https://www.r-bloggers.com/ :contentReferenceoaicite:17