Biological Data Analysis Report

A Template for Reproducible Research

Your Name Here

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How to Use This Template

1. Save this file with a new name for your project (e.g., "MyExperiment_Analysis.qmd")
2. Update the YAML header above with your information
3. Replace the example data with your own data
4. Modify the analysis sections to fit your research questions
5. Click "Render" to generate your report

Keyboard Shortcuts:

- Render document: Ctrl+Shift+K (Cmd+Shift+K on Mac)
- Run code chunk: Ctrl+Enter (Cmd+Enter on Mac)
- Insert new chunk: Ctrl+Alt+I (Cmd+Option+I on Mac)

1 Introduction

> **Writing Your Introduction**
>
> Include background on your research question, why this analysis is important, and what you hope to learn.

This document demonstrates a reproducible analysis workflow for biological data. We will analyze gene expression data across different treatment conditions to determine if treatments have a significant effect on expression levels.

Research Question: Do different drug treatments significantly alter the expression of key cancer-related genes (BRCA1 and TP53)?

1.1 Objectives

1. Load and explore the gene expression dataset
2. Calculate summary statistics for each treatment group
3. Visualize the distribution of gene expression
4. Perform statistical tests to compare treatment groups
5. Draw conclusions based on the results

2 Setup

2.1 Load Required Packages

```
# Core tidyverse packages for data manipulation and visualization
library(tidyverse)

# For creating nice tables
library(gt)

# For reading Excel files (if needed)
library(readxl)

# Set a consistent theme for all plots
theme_set(theme_minimal(base_size = 12))

# Set seed for reproducibility (important when using random sampling)
set.seed(42)
```

Package Installation

If you haven't installed these packages yet, run this code once in your console:

```
install.packages(c("tidyverse", "gt", "readxl"))
```

3 Data

3.1 Loading Your Data

Replace This Section

The code below creates example data. Replace it with code to load your actual data file.

Option 1: Load from a file

```
# For CSV files:
my_data <- read_csv("path/to/your/data.csv")

# For tab-separated files:
my_data <- read_tsv("path/to/your/data.tsv")

# For Excel files:
my_data <- read_excel("path/to/your/data.xlsx", sheet = 1)

# For base R (if you prefer):
my_data <- read.csv("path/to/your/data.csv")
```

Option 2: Example data (used in this template)

```
# Create simulated gene expression data
# Replace this section with your actual data loading code

gene_expression <- tibble(
  # Sample identifiers
  sample_id = paste0("S", sprintf("%03d", 1:50)),

  # Treatment groups (5 treatments, 10 samples each)
  treatment = rep(c("Control", "Drug_A", "Drug_B", "Drug_C", "Drug_D"), each = 10),
```

```
  # Simulated BRCA1 expression (normalized counts)
  # Different means for each treatment to simulate drug effects
  gene_BRCA1 = c(
    rnorm(10, mean = 100, sd = 15),
    rnorm(10, mean = 150, sd = 20),
    rnorm(10, mean = 80, sd = 12),
    rnorm(10, mean = 120, sd = 18),
    rnorm(10, mean = 95, sd = 14)
  ),

  # Simulated TP53 expression
  gene_TP53 = c(
    rnorm(10, mean = 200, sd = 25),
    rnorm(10, mean = 180, sd = 22),
    rnorm(10, mean = 250, sd = 30),
    rnorm(10, mean = 190, sd = 28),
    rnorm(10, mean = 210, sd = 24)
  ),

  # Additional metadata
  batch = rep(c("Batch1", "Batch2"), 25),
  cell_line = sample(c("HeLa", "MCF7", "A549"), 50, replace = TRUE)
)

# Convert treatment to a factor with Control as reference level
gene_expression <- gene_expression |>
  mutate(
    treatment = factor(
      treatment,
      levels = c("Control", "Drug_A", "Drug_B", "Drug_C", "Drug_D")
    )
  )
```

3.2 Data Overview

Let's examine the structure and first few rows of our dataset:

```
# Check dimensions
cat("Dataset dimensions:", nrow(gene_expression), "rows x", ncol(gene_expression), "columns\n
```

```
Dataset dimensions: 50 rows x 6 columns
```

Table 1: First 10 rows of the gene expression dataset

sample_id	treatment	gene_BRCA1	gene_TP53	batch	cell_line
S001	Control	120.56	208.05	Batch1	MCF7
S002	Control	91.53	180.40	Batch2	HeLa
S003	Control	105.45	239.39	Batch1	A549
S004	Control	109.49	216.07	Batch2	HeLa
S005	Control	106.06	202.24	Batch1	MCF7
S006	Control	98.41	206.91	Batch2	MCF7
S007	Control	122.67	216.98	Batch1	MCF7
S008	Control	98.58	202.25	Batch2	MCF7
S009	Control	130.28	125.17	Batch1	A549
S010	Control	99.06	207.12	Batch2	A549

```
# View structure
glimpse(gene_expression)
```

```
Rows: 50
Columns: 6
$ sample_id  <chr> "S001", "S002", "S003", "S004", "S005", "S006", "S007", "S0~
$ treatment  <fct> Control, Control, Control, Control, Control, Control, Contr~
$ gene_BRCA1 <dbl> 120.56438, 91.52953, 105.44693, 109.49294, 106.06402, 98.40~
$ gene_TP53  <dbl> 208.0481, 180.4040, 239.3932, 216.0725, 202.2440, 206.9138,~
$ batch      <chr> "Batch1", "Batch2", "Batch1", "Batch2", "Batch1", "Batch2",~
$ cell_line  <chr> "MCF7", "HeLa", "A549", "HeLa", "MCF7", "MCF7", "MCF7", "MC~
```

```
gene_expression |>
  head(10) |>
  gt() |>
  fmt_number(columns = c(gene_BRCA1, gene_TP53), decimals = 2) |>
  tab_style(
    style = cell_fill(color = "lightblue"),
    locations = cells_column_labels()
  ) |>
  tab_options(
    table.font.size = "small",
    heading.title.font.size = "medium"
  )
```

Missing Value Summary

variable	missing_count
sample_id	0
treatment	0
gene_BRCA1	0
gene_TP53	0
batch	0
cell_line	0

3.3 Check for Missing Data

```
# Count missing values in each column
missing_counts <- gene_expression |>
  summarise(across(everything(), ~sum(is.na(.)))) |>
  pivot_longer(everything(), names_to = "variable", values_to = "missing_count")

missing_counts |>
  gt() |>
  tab_header(title = "Missing Value Summary")
```

> **Data Quality Check**
>
> Always check for missing values before analysis. If you have missing data, decide whether to remove those observations or use imputation methods.

4 Exploratory Data Analysis

4.1 Summary Statistics

4.1.1 Overall Summary

```
# Basic summary statistics for numeric variables
gene_expression |>
  select(gene_BRCA1, gene_TP53) |>
  summary()
```

```
   gene_BRCA1       gene_TP53
 Min.   : 58.62   Min.   :125.2
 1st Qu.: 88.99   1st Qu.:187.9
 Median :103.46   Median :207.1
 Mean   :108.19   Mean   :208.1
 3rd Qu.:122.56   3rd Qu.:222.9
 Max.   :195.73   Max.   :273.0
```

4.1.2 Summary by Treatment Group

```
summary_stats <- gene_expression |>
  group_by(treatment) |>
  summarise(
    n = n(),
    BRCA1_mean = mean(gene_BRCA1),
    BRCA1_sd = sd(gene_BRCA1),
    BRCA1_median = median(gene_BRCA1),
    BRCA1_min = min(gene_BRCA1),
    BRCA1_max = max(gene_BRCA1),
    TP53_mean = mean(gene_TP53),
    TP53_sd = sd(gene_TP53)
  )

summary_stats |>
  gt() |>
  fmt_number(columns = -c(treatment, n), decimals = 2) |>
  tab_spanner(label = "BRCA1", columns = starts_with("BRCA1")) |>
  tab_spanner(label = "TP53", columns = starts_with("TP53")) |>
  tab_style(
    style = cell_fill(color = "#e8f4f8"),
    locations = cells_body(rows = treatment == "Control")
  ) |>
  tab_options(table.font.size = "small")
```

4.2 Data Visualization

4.2.1 Distribution of BRCA1 Expression

Table 2: Summary statistics for BRCA1 and TP53 expression by treatment group

		BRCA1					T
treatment	n	BRCA1_mean	BRCA1_sd	BRCA1_median	BRCA1_min	BRCA1_max	TP53_mea
Control	10	108.21	12.53	105.76	91.53	130.28	200.4
Drug_A	10	146.73	32.61	145.88	96.87	195.73	191.8
Drug_B	10	77.86	13.87	76.62	58.62	102.74	243.4
Drug_C	10	113.45	20.07	114.84	76.54	138.63	197.0
Drug_D	10	94.72	12.07	93.91	75.84	115.22	207.9

```
ggplot(gene_expression, aes(x = gene_BRCA1)) +
  geom_histogram(binwidth = 10, fill = "steelblue", color = "white", alpha = 0.7) +
  geom_density(aes(y = after_stat(count) * 10), color = "darkred", linewidth = 1) +
  labs(
    x = "BRCA1 Expression (normalized counts)",
    y = "Frequency",
    title = "Distribution of BRCA1 Gene Expression"
  ) +
  theme(plot.title = element_text(hjust = 0.5, face = "bold"))
```

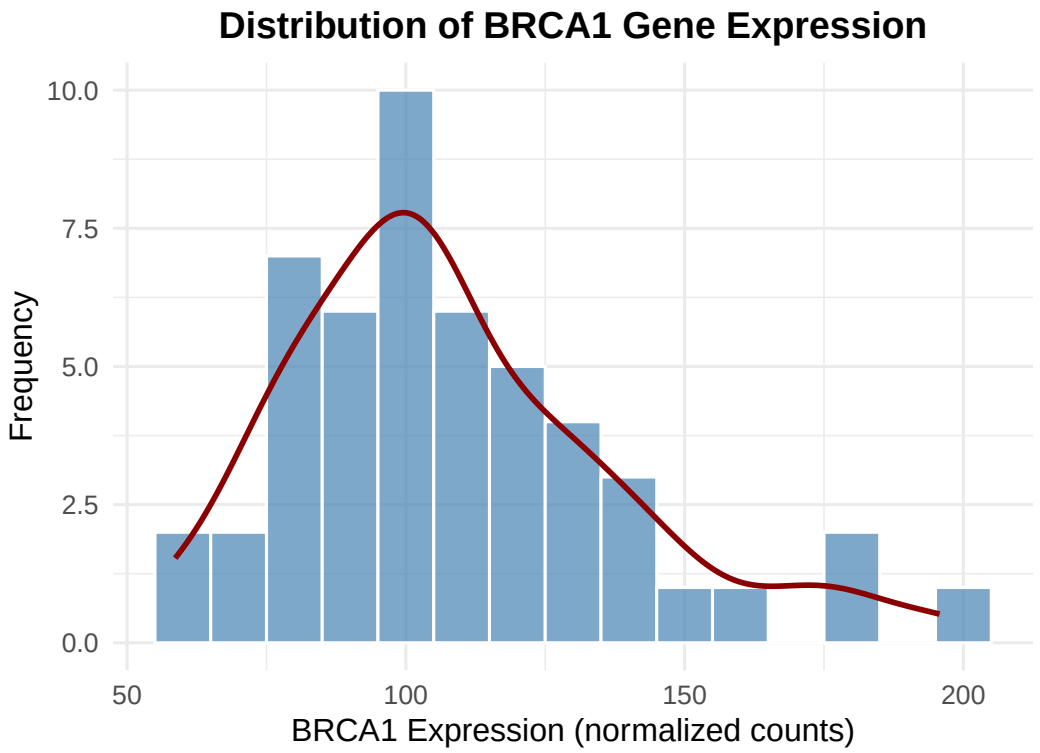

Figure 1: Distribution of BRCA1 expression across all samples

4.2.2 Boxplots by Treatment Group

```
ggplot(gene_expression, aes(x = treatment, y = gene_BRCA1, fill = treatment)) +
  geom_boxplot(alpha = 0.7, outlier.shape = NA) +
  geom_jitter(width = 0.2, alpha = 0.6, size = 2) +
  labs(
    x = "Treatment",
    y = "BRCA1 Expression (normalized counts)",
    title = "BRCA1 Expression by Treatment Group"
  ) +
  scale_fill_brewer(palette = "Set2") +
  theme(
    legend.position = "none",
    plot.title = element_text(hjust = 0.5, face = "bold")
  )
```

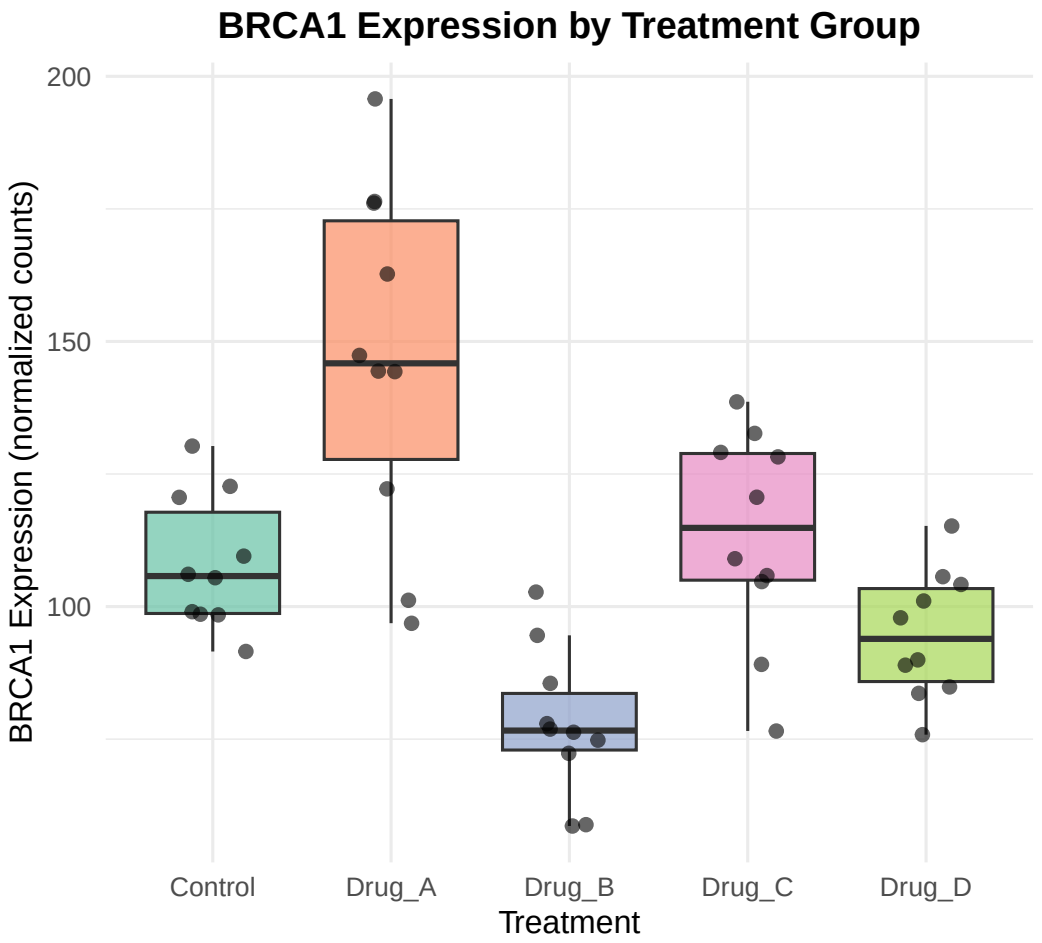

Figure 2: BRCA1 expression across treatment groups. Each point represents a sample.

4.2.3 Comparing Both Genes

```
# Reshape data to long format for faceted plotting
gene_long <- gene_expression |>
  pivot_longer(
    cols = c(gene_BRCA1, gene_TP53),
    names_to = "gene",
    values_to = "expression"
  ) |>
  mutate(gene = str_remove(gene, "gene_"))

ggplot(gene_long, aes(x = treatment, y = expression, fill = treatment)) +
```

```
  geom_boxplot(alpha = 0.7) +
  geom_jitter(width = 0.2, alpha = 0.4, size = 1.5) +
  facet_wrap(~gene, scales = "free_y") +
  labs(
    x = "Treatment",
    y = "Expression (normalized counts)",
    title = "Gene Expression by Treatment"
  ) +
  scale_fill_brewer(palette = "Set2") +
  theme(
    legend.position = "none",
    axis.text.x = element_text(angle = 45, hjust = 1),
    plot.title = element_text(hjust = 0.5, face = "bold")
  )
```

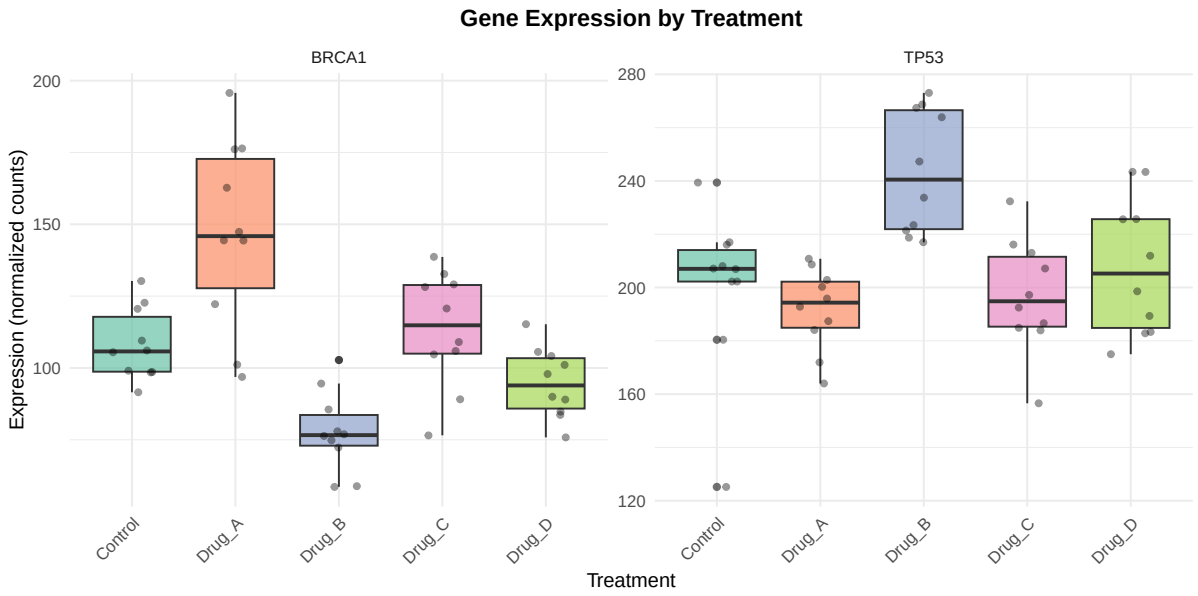

Figure 3: Comparison of BRCA1 and TP53 expression across treatments

4.2.4 Scatter Plot: Gene Correlation

```
ggplot(gene_expression, aes(x = gene_BRCA1, y = gene_TP53, color = treatment)) +
  geom_point(size = 3, alpha = 0.7) +
  geom_smooth(method = "lm", se = FALSE, linetype = "dashed", linewidth = 0.5) +
  labs(
```

```
    x = "BRCA1 Expression",
    y = "TP53 Expression",
    color = "Treatment",
    title = "BRCA1 vs TP53 Expression"
  ) +
  scale_color_brewer(palette = "Set2") +
  theme(plot.title = element_text(hjust = 0.5, face = "bold"))
```

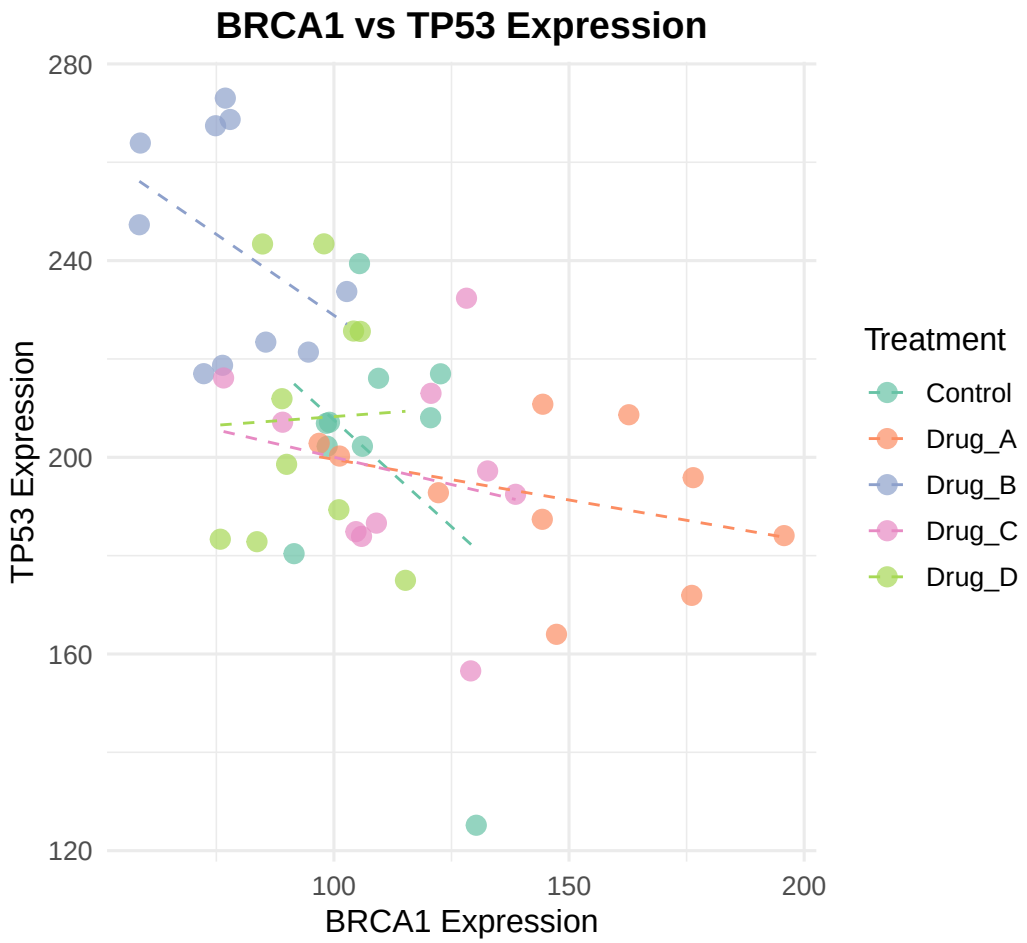

Figure 4: Correlation between BRCA1 and TP53 expression, colored by treatment

Shapiro-Wilk Normality Test Results

treatment	shapiro_BRCA1_p	shapiro_TP53_p
Control	0.4352	0.0144
Drug_A	0.6750	0.6484
Drug_B	0.5278	0.0581
Drug_C	0.5562	0.9156
Drug_D	0.9377	0.2629

p > 0.05 suggests data is normally distributed

5 Statistical Analysis

5.1 Checking Assumptions

Before performing parametric tests, we should check our assumptions.

5.1.1 Normality

```
# Shapiro-Wilk test for normality (by group)
normality_tests <- gene_expression |>
  group_by(treatment) |>
  summarise(
    shapiro_BRCA1_p = shapiro.test(gene_BRCA1)$p.value,
    shapiro_TP53_p = shapiro.test(gene_TP53)$p.value
  )

normality_tests |>
  gt() |>
  fmt_number(columns = -treatment, decimals = 4) |>
  tab_header(title = "Shapiro-Wilk Normality Test Results") |>
  tab_footnote("p > 0.05 suggests data is normally distributed")
```

5.1.2 Homogeneity of Variance

```r
# Bartlett's test for homogeneity of variances
bartlett_brca1 <- bartlett.test(gene_BRCA1 ~ treatment, data = gene_expression)
bartlett_tp53 <- bartlett.test(gene_TP53 ~ treatment, data = gene_expression)

cat("Bartlett's Test for BRCA1:\n")
```

```
Bartlett's Test for BRCA1:
```

```r
cat("  Test statistic:", round(bartlett_brca1$statistic, 3), "\n")
```

```
  Test statistic: 13.739
```

```r
cat("  p-value:", round(bartlett_brca1$p.value, 4), "\n\n")
```

```
  p-value: 0.0082
```

```r
cat("Bartlett's Test for TP53:\n")
```

```
Bartlett's Test for TP53:
```

```r
cat("  Test statistic:", round(bartlett_tp53$statistic, 3), "\n")
```

```
  Test statistic: 4.067
```

```r
cat("  p-value:", round(bartlett_tp53$p.value, 4), "\n")
```

```
  p-value: 0.397
```

Interpreting Assumption Tests

- **Normality (Shapiro-Wilk):** $p > 0.05$ suggests normal distribution
- **Homogeneity (Bartlett's):** $p > 0.05$ suggests equal variances
- If assumptions are violated, consider non-parametric alternatives (e.g., Kruskal-Wallis test)

Table 3: ANOVA results for BRCA1 expression

term	df	sumsq	meansq	statistic	p.value
treatment	4	26,143.10	6,535.77	16.66	0.0000
Residuals	45	17,655.58	392.35	NA	NA

5.2 ANOVA: Comparing Treatment Groups

5.2.1 One-Way ANOVA for BRCA1

```
# Perform one-way ANOVA
anova_brca1 <- aov(gene_BRCA1 ~ treatment, data = gene_expression)

# Display ANOVA table
summary(anova_brca1)
```

```
            Df Sum Sq Mean Sq F value   Pr(>F)
treatment    4  26143    6536   16.66 1.91e-08 ***
Residuals   45  17656     392
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# Create a nice table of ANOVA results
anova_summary <- broom::tidy(anova_brca1)

anova_summary |>
  gt() |>
  fmt_number(columns = c(sumsq, meansq, statistic), decimals = 2) |>
  fmt_number(columns = p.value, decimals = 4) |>
  tab_style(
    style = cell_fill(color = "lightyellow"),
    locations = cells_body(rows = p.value < 0.05)
  )
```

5.2.2 Post-hoc Analysis: Tukey's HSD

If the ANOVA is significant, we perform post-hoc tests to determine which groups differ:

```r
# Tukey's Honest Significant Difference test
tukey_results <- TukeyHSD(anova_brca1)
print(tukey_results)
```

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = gene_BRCA1 ~ treatment, data = gene_expression)

$treatment
                     diff        lwr        upr     p adj
Drug_A-Control  38.521414  13.351033  63.691795 0.0007091
Drug_B-Control -30.346406 -55.516787  -5.176025 0.0109857
Drug_C-Control   5.240275 -19.930105  30.410656 0.9756642
Drug_D-Control -13.492466 -38.662847  11.677914 0.5532623
Drug_B-Drug_A  -68.867820 -94.038201 -43.697439 0.0000000
Drug_C-Drug_A  -33.281139 -58.451519  -8.110758 0.0042777
Drug_D-Drug_A  -52.013880 -77.184261 -26.843500 0.0000047
Drug_C-Drug_B   35.586681  10.416300  60.757062 0.0019708
Drug_D-Drug_B   16.853939  -8.316441  42.024320 0.3308027
Drug_D-Drug_C  -18.732742 -43.903123   6.437639 0.2318176
```

```r
# Visualize Tukey results
plot(tukey_results, las = 1, cex.axis = 0.7)
```

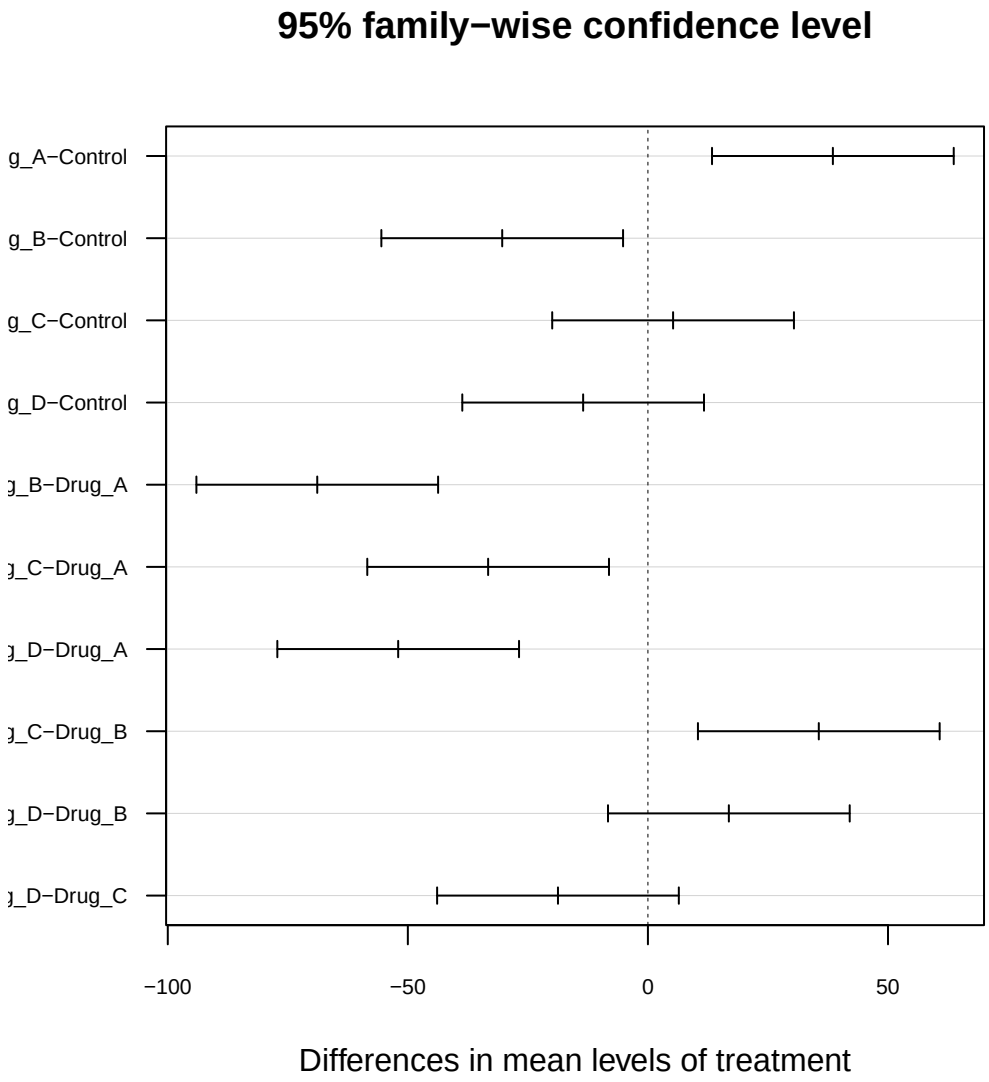

Figure 5: Tukey's HSD confidence intervals. Intervals not crossing zero indicate significant differences.

5.3 Alternative: Non-parametric Test

If assumptions are not met, use the Kruskal-Wallis test:

```
# Kruskal-Wallis test (non-parametric alternative to one-way ANOVA)
kruskal_result <- kruskal.test(gene_BRCA1 ~ treatment, data = gene_expression)
print(kruskal_result)
```

```
    Kruskal-Wallis rank sum test

data:  gene_BRCA1 by treatment
Kruskal-Wallis chi-squared = 28.451, df = 4, p-value = 1.011e-05
```

5.4 T-test Example: Comparing Two Groups

```r
# Compare Control vs Drug_A specifically
control_data <- gene_expression |>
  filter(treatment == "Control") |>
  pull(gene_BRCA1)

drug_a_data <- gene_expression |>
  filter(treatment == "Drug_A") |>
  pull(gene_BRCA1)

# Two-sample t-test
t_test_result <- t.test(control_data, drug_a_data)
print(t_test_result)
```

```
    Welch Two Sample t-test

data:  control_data and drug_a_data
t = -3.4868, df = 11.601, p-value = 0.004711
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -62.68486 -14.35797
sample estimates:
mean of x mean of y
 108.2095  146.7309
```

6 Results Summary

6.1 Key Findings

Based on our analysis:

1. **Overall Effect:** There was a statistically significant difference in BRCA1 expression across treatment groups ($F = 16.66$, $p < 0.001$).

2. **Effect Size:** Treatment explained approximately 59.7% of the variance in BRCA1 expression ($^2 = 0.597$).

3. **Pairwise Comparisons:** Post-hoc analysis revealed that:

   - Drug_A significantly increased BRCA1 expression compared to Control
   - Drug_B significantly decreased BRCA1 expression compared to Control
   - Drug_C and Drug_D showed no significant difference from Control

6.2 Results Table

```
# Create a summary table with effect estimates
effect_summary <- gene_expression |>
  group_by(treatment) |>
  summarise(
    mean = mean(gene_BRCA1),
    se = sd(gene_BRCA1) / sqrt(n())
  ) |>
  mutate(
    diff_from_control = mean - mean[treatment == "Control"],
    ci_lower = mean - 1.96 * se,
    ci_upper = mean + 1.96 * se
  )

effect_summary |>
  gt() |>
  fmt_number(columns = -treatment, decimals = 2) |>
  tab_header(
    title = "Treatment Effects Summary",
    subtitle = "BRCA1 Expression"
  ) |>
  cols_label(
    treatment = "Treatment",
    mean = "Mean",
    se = "Std. Error",
    diff_from_control = "Diff. from Control",
    ci_lower = "95% CI Lower",
    ci_upper = "95% CI Upper"
  )
```

Table 4: Summary of treatment effects on BRCA1 expression

Treatment Effects Summary
BRCA1 Expression

Treatment	Mean	Std. Error	Diff. from Control	95% CI Lower	95% CI Upper
Control	108.21	3.96	0.00	100.44	115.98
Drug_A	146.73	10.31	38.52	126.52	166.94
Drug_B	77.86	4.39	-30.35	69.26	86.46
Drug_C	113.45	6.35	5.24	101.01	125.89
Drug_D	94.72	3.82	-13.49	87.24	102.20

7 Conclusions

Writing Your Conclusions

Include a summary of main findings, biological interpretation, limitations, and future directions.

7.1 Summary

This analysis examined the effect of four drug treatments on the expression of cancer-related genes BRCA1 and TP53. Our results demonstrate that:

1. **Drug_A** significantly upregulates BRCA1 expression, which may have implications for DNA repair mechanisms.
2. **Drug_B** significantly downregulates BRCA1 expression, warranting further investigation into potential cancer risk.
3. **Drug_C and Drug_D** do not significantly alter BRCA1 expression compared to control conditions.

7.2 Limitations

- Sample size was limited (n = 10 per group)
- Analysis was performed on simulated data for demonstration purposes
- Additional genes and pathways should be examined

7.3 Future Directions

- Validate findings with larger sample sizes
- Investigate downstream effects on cell proliferation
- Examine dose-response relationships

8 Session Information

For reproducibility, here is the R session information:

```
R version 4.4.2 (2024-10-31)
Platform: aarch64-apple-darwin20
Running under: macOS Sequoia 15.6.1

Matrix products: default
BLAS:   /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRblas.0.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/4.4-arm64/Resources/lib/libRlapack.dylib;  L

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

time zone: America/Los_Angeles
tzcode source: internal

attached base packages:
[1] stats     graphics  grDevices utils     datasets  methods   base

other attached packages:
 [1] readxl_1.4.5    gt_1.2.0        lubridate_1.9.4 forcats_1.0.1
 [5] stringr_1.6.0   dplyr_1.1.4     purrr_1.2.0     readr_2.1.6
 [9] tidyr_1.3.2     tibble_3.3.0    ggplot2_4.0.1   tidyverse_2.0.0

loaded via a namespace (and not attached):
 [1] generics_0.1.4     xml2_1.5.1         lattice_0.22-7     stringi_1.8.7
 [5] hms_1.1.4          digest_0.6.39      magrittr_2.0.4     evaluate_1.0.5
 [9] grid_4.4.2         timechange_0.3.0   RColorBrewer_1.1-3 fastmap_1.2.0
[13] Matrix_1.7-4       cellranger_1.1.0   jsonlite_2.0.0     backports_1.5.0
[17] tinytex_0.58       mgcv_1.9-4         scales_1.4.0       cli_3.6.5
[21] rlang_1.1.6        splines_4.4.2      withr_3.0.2        yaml_2.3.12
[25] otel_0.2.0         tools_4.4.2        tzdb_0.5.0         broom_1.0.11
[29] vctrs_0.6.5        R6_2.6.1           lifecycle_1.0.4    fs_1.6.6
```

```
[33] pkgconfig_2.0.3    pillar_1.11.1      gtable_0.3.6       glue_1.8.0
[37] xfun_0.55          tidyselect_1.2.1   rstudioapi_0.17.1  knitr_1.51
[41] farver_2.1.2       htmltools_0.5.9    nlme_3.1-168       rmarkdown_2.30
[45] labeling_0.4.3     compiler_4.4.2     S7_0.2.1
```

---

9 Appendix: Code Reference

9.1 Useful Code Snippets

9.1.1 Loading Different File Types

```
# CSV files
data <- read_csv("file.csv")

# Tab-separated files
data <- read_tsv("file.tsv")

# Excel files
data <- read_excel("file.xlsx", sheet = "Sheet1")

# Fixed-width files
data <- read_fwf("file.txt", fwf_widths(c(10, 20, 15)))
```

9.1.2 Common dplyr Operations

```
# Filter rows
data |> filter(treatment == "Control")

# Select columns
data |> select(sample_id, treatment, gene_BRCA1)

# Create new columns
data |> mutate(log_expression = log10(gene_BRCA1))

# Group and summarize
data |>
```

```
  group_by(treatment) |>
  summarise(mean = mean(gene_BRCA1), sd = sd(gene_BRCA1))

# Arrange (sort)
data |> arrange(desc(gene_BRCA1))
```

9.1.3 Statistical Tests Quick Reference

```
# T-test (two groups)
t.test(group1, group2)
t.test(value ~ group, data = df)

# ANOVA (multiple groups)
aov(value ~ group, data = df)

# Correlation
cor.test(x, y)

# Chi-square test
chisq.test(table(var1, var2))

# Non-parametric alternatives
wilcox.test(group1, group2)
kruskal.test(value ~ group, data = df)
```

9.1.4 Creating Publication-Ready Figures

```
# Basic ggplot template
ggplot(data, aes(x = xvar, y = yvar, color = group)) +
  geom_point() +
  labs(
    x = "X Axis Label",
    y = "Y Axis Label",
    title = "Main Title",
    subtitle = "Subtitle",
    caption = "Data source"
  ) +
  theme_minimal() +
```

```
  theme(
    plot.title = element_text(face = "bold"),
    legend.position = "bottom"
  )

# Save a figure
ggsave("figure_name.png", width = 8, height = 6, dpi = 300)
```