R final submission

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Final Project

Before class on 8/21, you should submit (1) your compiled PDF from LaTeX, (2) your corresponding .tex file, (3) a knitted PDF of the R markdown file with your code for the final submission, and (4) a link to your now public facing GitHub repository.

```
#Final subission
#Load gene expression data
gene_data <- read.csv(file = "QBS103_GSE157103_genes.csv",row.names=1)

#Load metadata for participants
series <- read.csv(file = "QBS103_GSE157103_series_matrix-1.csv")
```

Generate a table formatted in LaTeX of summary statistics for all the covariates you looked at and 2 additional continuous (3 total) and 1 additional categorical variable (3 total). (5 pts) Stratifying by one of your categorical variables Tables should report n (%) for categorical variables Tables should report mean (sd) or median [IQR] for continuous variables

```
only_num <- function(x) {
  is_num <- grepl("^[[:space:]]*[0-9]+(?:\\.[0-9]+)?[[:space:]]*$", x)
  out <- rep(NA_real_, length(x))
  out[is_num] <- as.numeric(trimws(x[is_num]))
  out
}

df <- series %>%
  mutate(
    # Remove any extra spaces from 'sex' variable
    sex = trimws(sex),
    # Recode ICU status to standardized labels
    icu_status = ifelse(tolower(trimws(icu_status)) == "yes", "ICU", "NonICU"),
    # Convert 'age' to numeric
    age = only_num(age),
    # Convert ferritin to numeric (ignore warnings if conversion fails)
    ferritin = only_num(ferritin.ng.ml.),
    # Convert CRP to numeric (ignore warnings)
    crp = only_num(crp.mg.l.),
    # Recode mechanical ventilation variable
    mech_vent = ifelse(trimws(mechanical_ventilation) == "yes", "Yes", "No")
  )
```

```r
# Summarize categorical variables (sex and mechanical ventilation) by ICU status
df %>%
  group_by(icu_status) %>%
  summarise(
    n_sex_female = sum(sex == "female", na.rm=TRUE),    # Count number of females
    n_sex_male   = sum(sex == "male", na.rm=TRUE),      # Count number of males
    n_mechvent_yes = sum(mech_vent == "Yes", na.rm=TRUE), # Count patients with mechanical ventilation
    n_mechvent_no  = sum(mech_vent == "No", na.rm=TRUE),  # Count patients without mechanical ventilati
    .groups="drop"
  )
```

```
## # A tibble: 2 x 5
##   icu_status n_sex_female n_sex_male n_mechvent_yes n_mechvent_no
##   <chr>             <int>      <int>          <int>         <int>
## 1 ICU                  24         41             46            20
## 2 NonICU               27         33              5            55
```

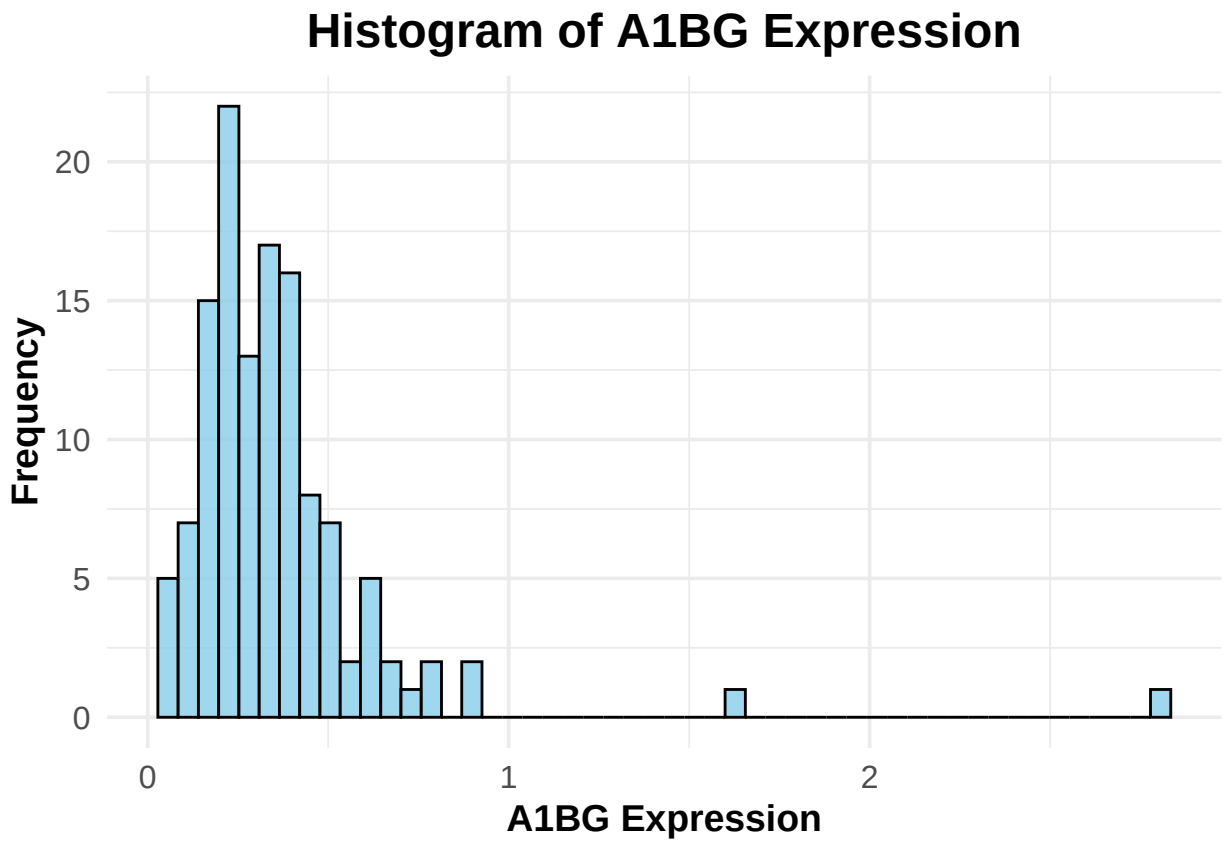
```r
# Summarize continuous variables (age, ferritin, CRP) by ICU status
df %>%
  group_by(icu_status) %>%
  summarise(
    age_mean = mean(age, na.rm=TRUE),    # Calculate mean age
    age_sd   = sd(age, na.rm=TRUE),      # Calculate standard deviation of age
    ferr_median = median(ferritin, na.rm=TRUE),    # Median ferritin level
    ferr_IQR1 = quantile(ferritin, 0.25, na.rm=TRUE), # 25th percentile (Q1) of ferritin
    ferr_IQR3 = quantile(ferritin, 0.75, na.rm=TRUE), # 75th percentile (Q3) of ferritin
    crp_mean = mean(crp, na.rm=TRUE),    # Mean CRP level
    crp_sd   = sd(crp, na.rm=TRUE)       # Standard deviation of CRP
  )
```

```
## # A tibble: 2 x 8
##   icu_status age_mean age_sd ferr_median ferr_IQR1 ferr_IQR3 crp_mean crp_sd
##   <chr>         <dbl>  <dbl>       <dbl>     <dbl>     <dbl>    <dbl>  <dbl>
## 1 ICU            63.5   14.0         685       325      1212     150.  106.
## 2 NonICU         58.7   17.8         401       131       870     109.   94.4
```

Generate final a publication quality histogram, scatter plot, and boxplot from submission 1 (i.e. only for your first gene of interest) (5 pts)

```r
#1.
#Histogram of gene expression
#Select the first gene (A1BG) for analysis and convert to numeric vector
new_gene <- gene_data[1, ]

#Plot histogram for A1BG expression values across all participants
ggplot(data.frame(value = as.numeric(new_gene)), aes(x = value)) +
  geom_histogram(bins = 50, color = "black", fill = "skyblue", alpha = 0.8) +
  labs(
    title = "Histogram of A1BG Expression",
    x = "A1BG Expression",
    y = "Frequency"
  ) +
```

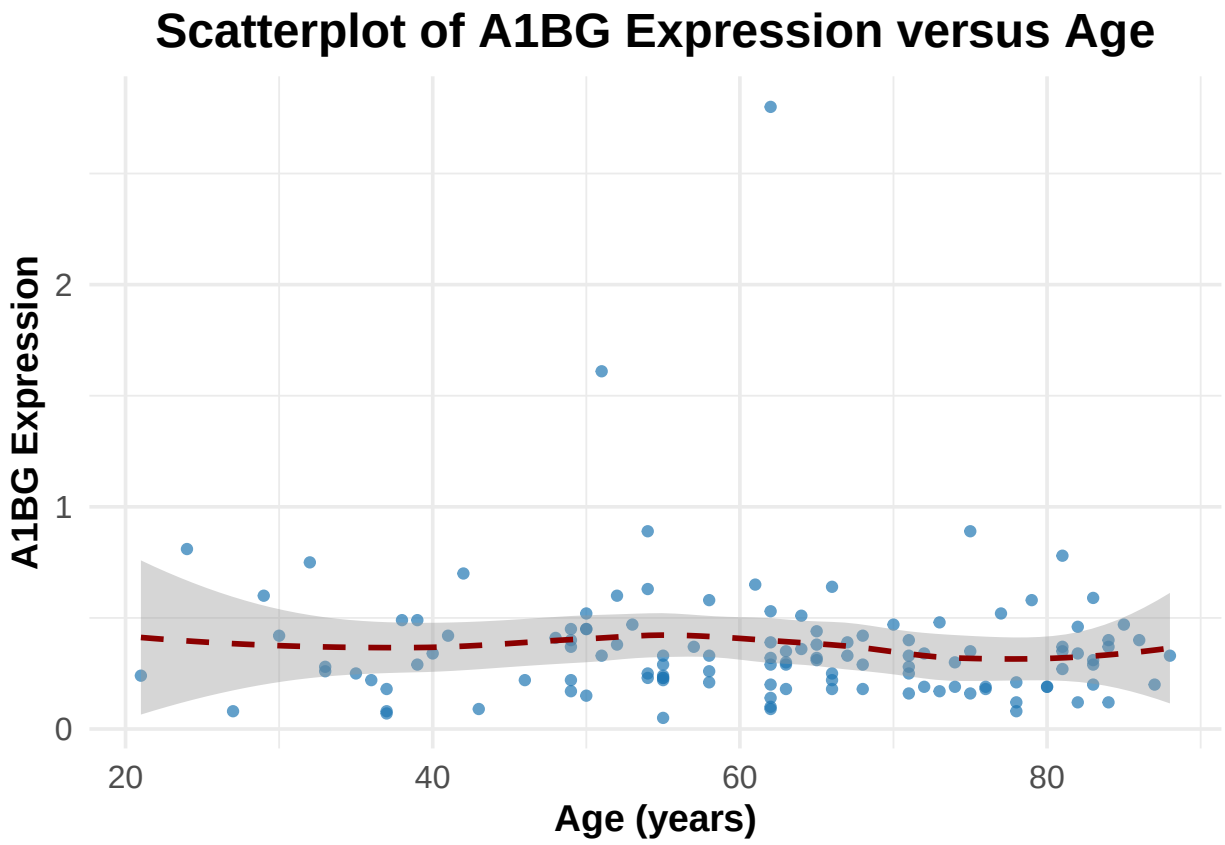
```
  theme_minimal(base_size = 14) +
  theme(
    plot.title = element_text(size = 18, face = "bold", hjust = 0.5),
    axis.title = element_text(size = 14, face = "bold"),
    axis.text = element_text(size = 12)
  )
```




```
#2.
#Scatterplot of gene expression vs age
#Pivot gene expression row into long format for merging
gene_line1 <- new_gene %>%
  pivot_longer(cols = everything(),names_to = "participant_id",values_to = "A1BG_value")

# Merge with metadata by participant_id to obtain age and other covariates
new_df<-merge(series,gene_line1,by="participant_id")

#Prepare age levels for the x-axis: numeric ages in ascending order, then special categories
num_ages <- sort(as.numeric(unique(new_df$age)[!grepl("[^0-9]", unique(new_df$age))]))
special_ages <- unique(new_df$age)[grepl("[^0-9]", unique(new_df$age))]
age_levels <- c(as.character(num_ages), special_ages)

# Scatterplot: A1BG expression vs age (x-axis shows all ages in specified order)
new_df <- new_df %>%
  mutate(age_num = suppressWarnings(as.numeric(trimws(age)))) %>%  # avoid coercion warnings
  filter(is.finite(age_num), is.finite(A1BG_value))                # drop NAs/Inf before ggplot
```

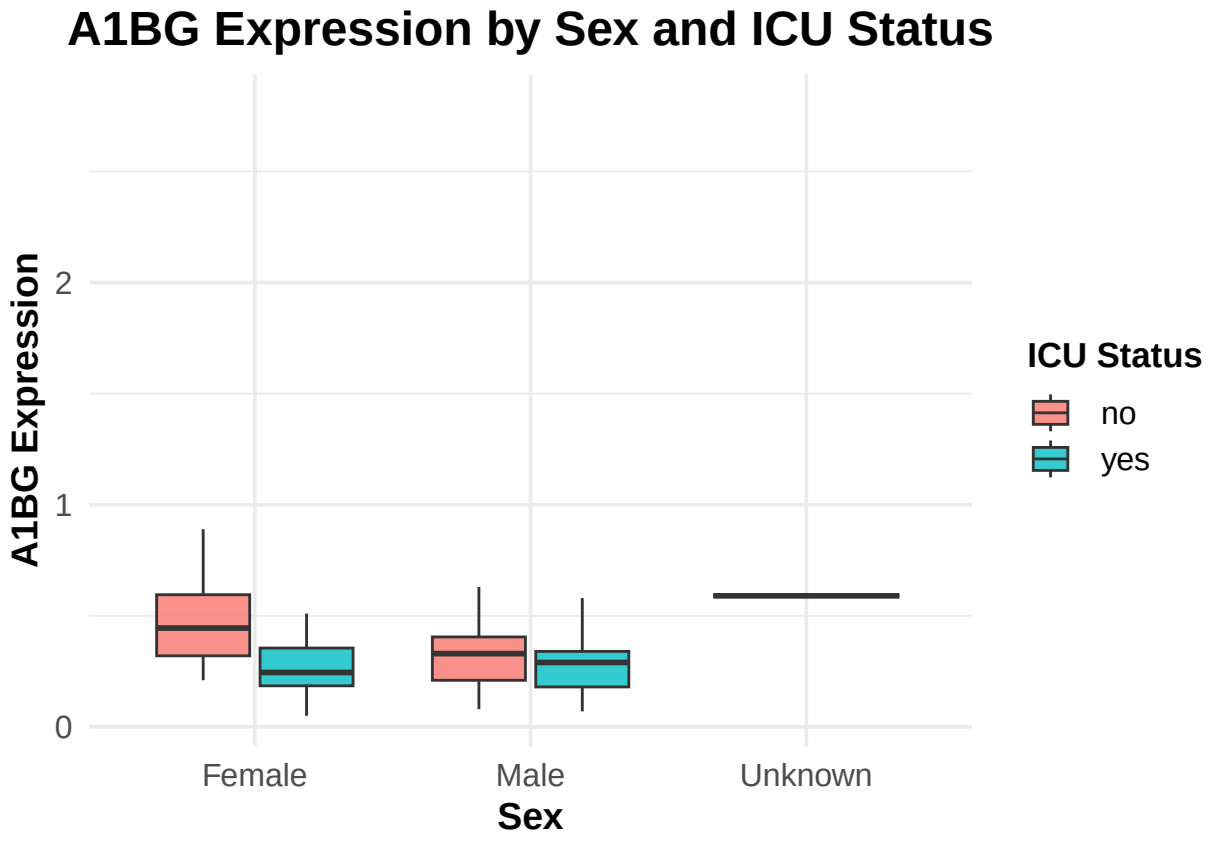
```
ggplot(new_df, aes(x = age_num, y = A1BG_value)) +
  geom_point(alpha = 0.7, color = "#1F77B4") +
  geom_smooth(method = "loess", se = TRUE, color = "darkred", linetype = "dashed") +
  labs(
    title = "Scatterplot of A1BG Expression versus Age",
    x = "Age (years)",
    y = "A1BG Expression"
  ) +
  theme_minimal(base_size = 14) +
  theme(
    plot.title = element_text(size = 18, face = "bold", hjust = 0.5),
    axis.title = element_text(size = 14, face = "bold"),
    axis.text = element_text(size = 12)
  )
```

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




```
#3.
#Boxplot of gene expression by sex and ICU status
#sex: categorical variable; icu_status: categorical variable
new_df <- new_df %>%
  mutate(
    sex = tolower(trimws(sex)),
    sex = recode(sex,
```

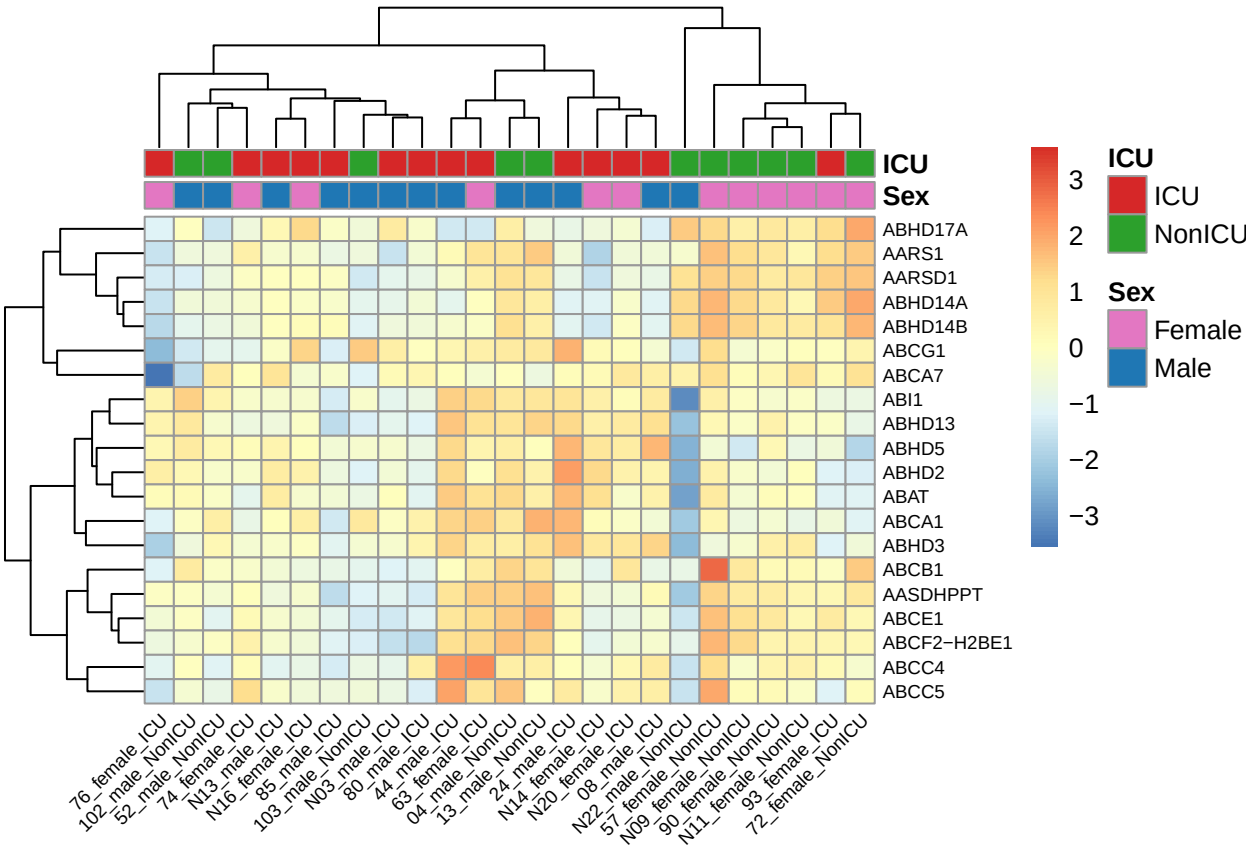
```
                  "female" = "Female",
                  "male"   = "Male",
                  "unknown"= "Unknown"),
    sex = factor(sex, levels = c("Female","Male","Unknown"))
  )

ggplot(new_df, aes(x = sex, y = A1BG_value, fill = icu_status)) +
  geom_boxplot(alpha = 0.8, outlier.shape = NA) +
  labs(
    title = "A1BG Expression by Sex and ICU Status",
    x = "Sex",
    y = "A1BG Expression",
    fill = "ICU Status"
  ) +
  theme_minimal(base_size = 14) +
  theme(
    plot.title = element_text(size = 18, face = "bold", hjust = 0.5),
    axis.title = element_text(size = 14, face = "bold"),
    axis.text = element_text(size = 12),
    legend.title = element_text(size = 13, face = "bold"),
    legend.text = element_text(size = 12)
  )
```



Generate a heatmap (5 pts) Heatmap should include at least 10 genes Include tracking bars for the 2 categorical covariates in your boxplot Heatmaps should include clustered rows and columns

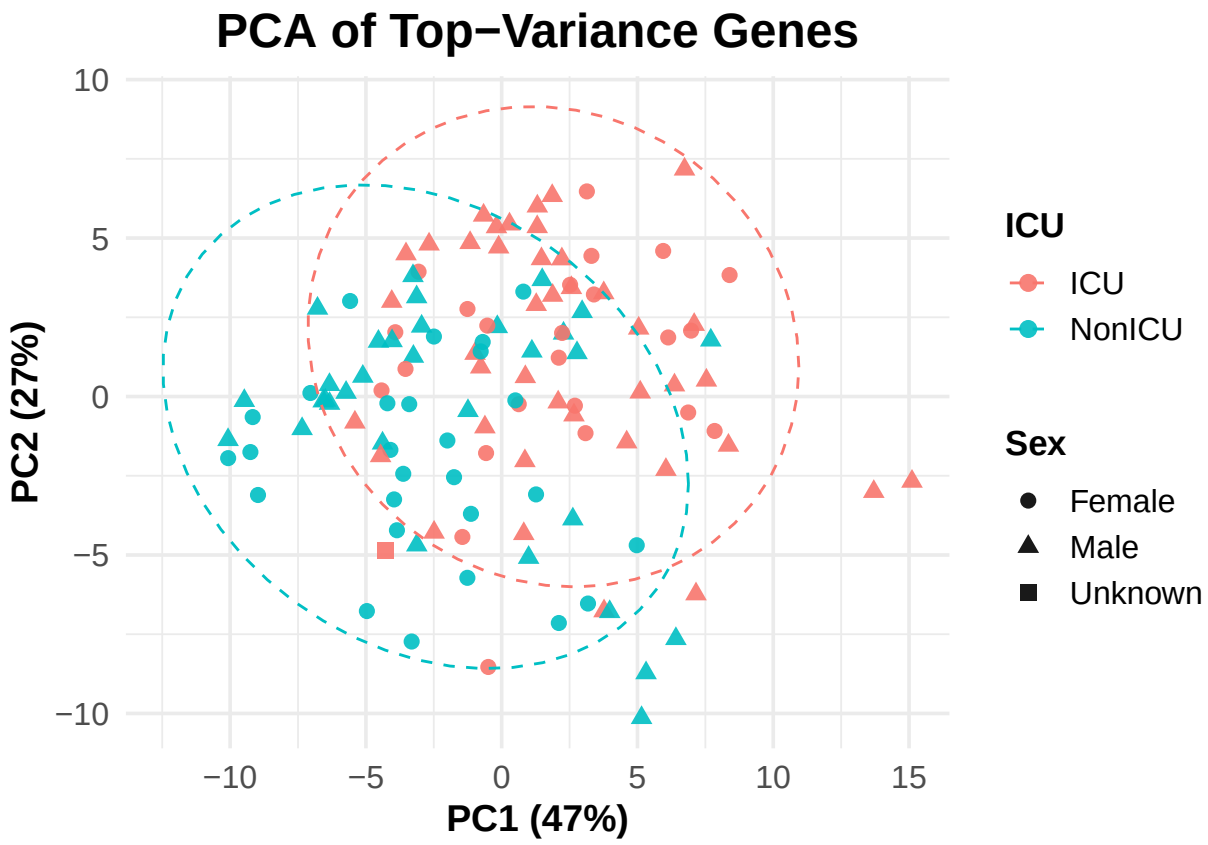
```r
# Convert gene expression data into a numeric matrix
mat <- as.matrix(gene_data)

# Find overlapping participant IDs between gene expression and metadata
common <- intersect(colnames(mat), series$participant_id)

# Keep only common participants in the gene expression matrix
mat <- mat[, common, drop = FALSE]

# Subset metadata for the same participants and align the order
series_sub <- series %>% filter(participant_id %in% common) %>%
  arrange(match(participant_id, colnames(mat)))

# Log2-transform the expression values to stabilize variance
mat_log <- log2(mat + 1)

# Select the top 20 most variable genes across samples
topN <- 20
top_idx <- order(apply(mat_log, 1, var, na.rm = TRUE), decreasing = TRUE)[1:topN]
sub <- mat_log[top_idx, , drop = FALSE]

# Clean up metadata variables: sex and ICU status
sex_clean <- trimws(series_sub$sex)
icu_raw   <- trimws(series_sub$icu_status)

# Convert ICU status to binary factor
icu_clean <- ifelse(tolower(icu_raw) == "yes", "ICU", "NonICU")

# Convert sex into male/female categories
sex_clean <- ifelse(grepl("^f", tolower(sex_clean)), "Female", "Male")

# Prepare annotation dataframe for heatmap
annotationData <- data.frame(
  Sex = factor(sex_clean, levels = c("Female","Male")),
  ICU = factor(icu_clean, levels = c("ICU","NonICU")),
  row.names = series_sub$participant_id
)

# Define color scheme for annotations
annotationColors <- list(
  Sex = c(Female = "#E377C2", Male = "#1F77B4"),
  ICU = c(ICU = "#D62728", NonICU = "#2CA02C")
)

# Randomly select 25 samples for visualization
set.seed(100)
subset_cols <- sample(colnames(sub), 25)
sub25 <- sub[, subset_cols, drop = FALSE]
ann25 <- annotationData[subset_cols, , drop = FALSE]

# Shorten column labels for readability (keep ID, sex, and ICU status)
short_labels <- ifelse(
  grepl("^COVID", colnames(sub25)),
```

```
  sub("^COVID_(\\d+)_\\d+y_([^_]+)_(ICU|NonICU)$",
      "\\1_\\2_\\3", colnames(sub25)),
  sub("^NONCOVID_(\\d+)_\\d+y_([^_]+)_(ICU|NonICU)$",
      "N\\1_\\2_\\3", colnames(sub25))
)

# Draw heatmap of top variable genes with sample annotations
pheatmap(
  sub25,
  scale = "row",                            # scale genes across samples
  clustering_distance_cols = "euclidean", # distance metric for columns
  clustering_distance_rows = "euclidean", # distance metric for rows
  clustering_method = "complete",           # hierarchical clustering method
  annotation_col = ann25,                   # add sample metadata as annotation
  annotation_colors = annotationColors,     # use predefined colors
  show_colnames = TRUE,                     # display sample labels
  labels_col = short_labels,                # use shortened labels
  fontsize_col = 7,                         # font size for column labels
  angle_col = 45,                           # rotate labels for clarity
  fontsize_row = 7,                         # font size for row labels
)
```



Going through the documentation for ggplot2, generate a plot type that we did not previously discuss in class that describes your data in a new and unique way (5 pts)

```r
# Select top N genes with highest variance across samples
topN <- 50
top_idx <- order(apply(mat_log, 1, var, na.rm = TRUE), decreasing = TRUE)[1:topN]
mat_top <- mat_log[top_idx, , drop = FALSE]

# PCA on samples (transpose), centered & scaled
pca <- prcomp(t(mat_top), center = TRUE, scale. = TRUE)

# First two PCs to data frame
pca_df <- as.data.frame(pca$x[, 1:2])
pca_df$participant_id <- rownames(pca_df)

# Clean metadata; standardize labels
meta <- series_sub %>%
  mutate(
    sex = tolower(trimws(sex)),
    sex = ifelse(sex %in% c("female","male"), sex, "unknown"),
    icu_status = ifelse(tolower(trimws(icu_status)) == "yes", "ICU", "NonICU")
  ) %>%
  select(participant_id, sex, icu_status)

# Join and coerce to factors (good for legends/shapes)
pca_df <- dplyr::left_join(pca_df, meta, by = "participant_id") %>%
  mutate(
    sex = recode(sex, "female" = "Female", "male" = "Male", "unknown" = "Unknown"),
    sex = factor(sex, levels = c("Female","Male","Unknown")),
    icu_status = factor(icu_status, levels = c("ICU","NonICU"))
  )

# Variance explained for axis labels
var_exp <- round(100 * (pca$sdev^2 / sum(pca$sdev^2)))[1:2]

# Plot: map color globally, shape only on points (no warning from stat_ellipse)
ggplot(pca_df, aes(PC1, PC2, color = icu_status)) +
  geom_point(aes(shape = sex), size = 2.5, alpha = 0.9) +
  stat_ellipse(aes(group = icu_status), linetype = 2) +
  labs(
    title = "PCA of Top-Variance Genes",
    x = paste0("PC1 (", var_exp[1], "%)"),
    y = paste0("PC2 (", var_exp[2], "%)"),
    color = "ICU", shape = "Sex"
  ) +
  theme_minimal(base_size = 14) +
  theme(
    plot.title = element_text(size = 18, face = "bold", hjust = 0.5),
    axis.title = element_text(size = 14, face = "bold"),
    axis.text = element_text(size = 12),
    legend.title = element_text(size = 13, face = "bold"),
    legend.text = element_text(size = 12)
  )
```


PCA of Top−Variance Genes
PC2 (27%)
PC1 (47%)
10
5
0
−5
−10
−10
−5
0
5
10
15
ICU
ICU
NonICU
Sex
Female
Male
Unknown