Analysis of Nasal Epithelium

2024-09-27

R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see http://rmarkdown.rstudio.com (http://rmarkdown.rstudio.com).

When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

```
#install.packages("limma")
library(limma)
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
```

```
library(tidyverse)
```

```
## ── Attaching core tidyverse packages ──────────────────────── tidyverse 2.0.0 ──
## ✔ forcats   1.0.0     ✔ readr     2.1.5
## ✔ ggplot2   3.5.1     ✔ stringr   1.5.1
## ✔ lubridate 1.9.3     ✔ tibble    3.2.1
## ✔ purrr     1.0.2     ✔ tidyr     1.3.1
```

```
## ── Conflicts ────────────────────────────────────────── tidyverse_conflicts() ──
## ✖ dplyr::filter() masks stats::filter()
## ✖ dplyr::lag()    masks stats::lag()
## ℹ Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts
to become errors
```

```
library(GEOquery)
```

```
## Loading required package: Biobase
## Loading required package: BiocGenerics
##
## Attaching package: 'BiocGenerics'
##
## The following objects are masked from 'package:lubridate':
##
##     intersect, setdiff, union
##
## The following objects are masked from 'package:dplyr':
##
##     combine, intersect, setdiff, union
##
## The following object is masked from 'package:limma':
##
##     plotMA
##
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
##
## The following objects are masked from 'package:base':
##
##     anyDuplicated, aperm, append, as.data.frame, basename, cbind,
##     colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
##     get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
##     match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
##     Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which.max, which.min
##
## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.
##
## Setting options('download.file.method.GEOquery'='auto')
## Setting options('GEOquery.inmemory.gpl'=FALSE)
```

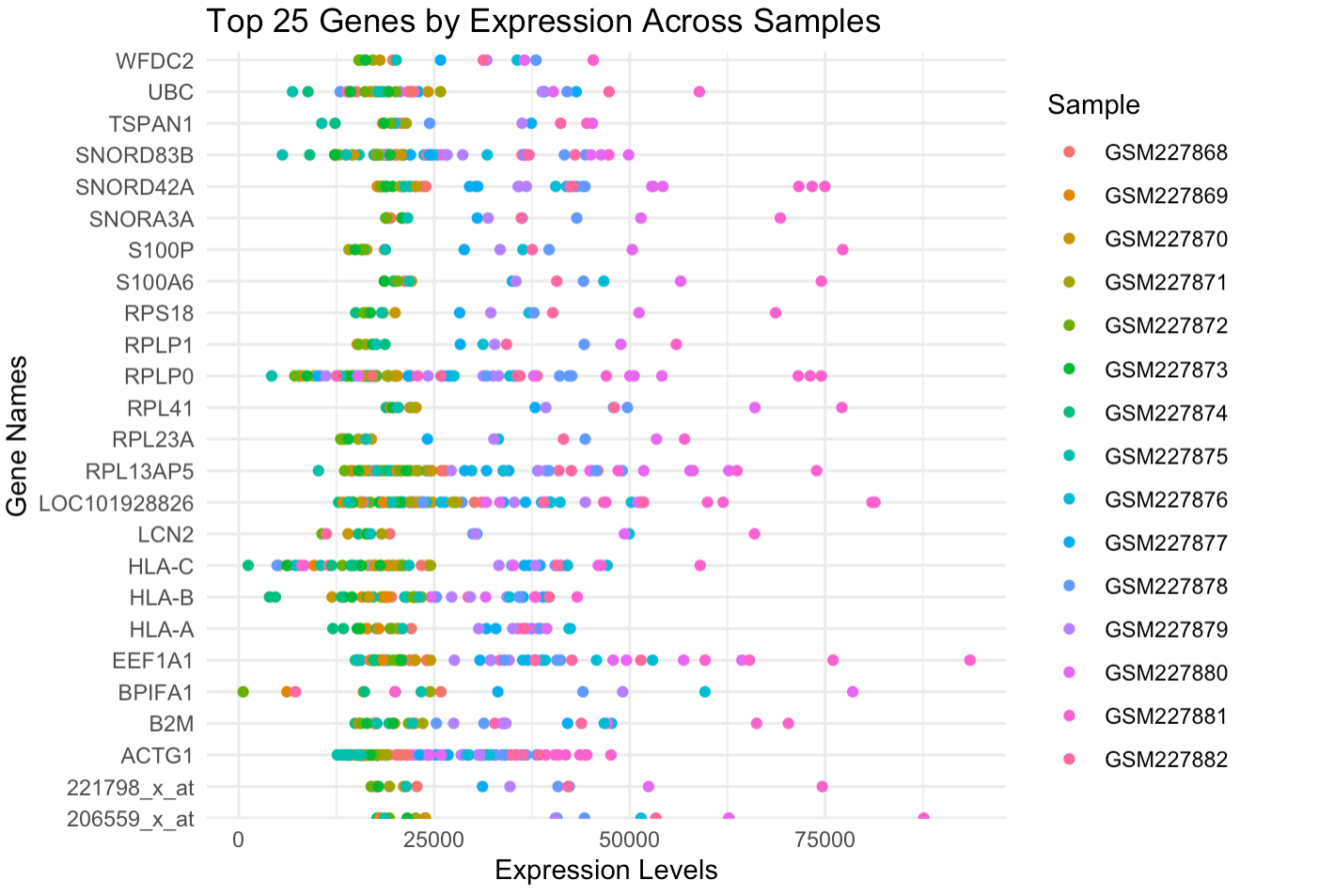
```
library(pheatmap)


file_path <- "~/Downloads/GDS3309_full.soft"
raw_data <- readLines(file_path)
head(raw_data, 100)
```

```
##   [1] "^DATABASE = Geo"
##   [2] "!Database_name = Gene Expression Omnibus (GEO)"
##   [3] "!Database_institute = NCBI NLM NIH"
##   [4] "!Database_web_link = http://www.ncbi.nlm.nih.gov/geo"
##   [5] "!Database_email = geo@ncbi.nlm.nih.gov"
##   [6] "!Database_ref = Nucleic Acids Res. 2005 Jan 1;33 Database Issue:D562-6"
##   [7] "^DATASET = GDS3309"
##   [8] "!dataset_title = Cigarette smoking effect on the nasal epithelium"
##   [9] "!dataset_description = Analysis of nasal epithelia from cigarette smokers. Cig
arette smoke creates a field of injury in epithelial cells lining the respiratory tract.
Results extend the concept of a smoking-induced field of injury beyond intrathoracic (br
onchial) epithelia to extrathoracic epithelia that line the nose."
##  [10] "!dataset_type = Expression profiling by array"
##  [11] "!dataset_pubmed_id = 18513428"
##  [12] "!dataset_platform = GPL571"
##  [13] "!dataset_platform_organism = Homo sapiens"
##  [14] "!dataset_platform_technology_type = in situ oligonucleotide"
##  [15] "!dataset_feature_count = 22277"
##  [16] "!dataset_sample_organism = Homo sapiens"
##  [17] "!dataset_sample_type = RNA"
##  [18] "!dataset_channel_count = 1"
##  [19] "!dataset_sample_count = 15"
##  [20] "!dataset_value_type = count"
##  [21] "!dataset_reference_series = GSE8987"
##  [22] "!dataset_order = none"
##  [23] "!dataset_update_date = Sep 10 2008"
##  [24] "^SUBSET = GDS3309_1"
##  [25] "!subset_dataset_id = GDS3309"
##  [26] "!subset_description = control"
##  [27] "!subset_sample_id = GSM227868,GSM227870,GSM227871,GSM227874,GSM227876,GSM22787
7,GSM227878,GSM227880"
##  [28] "!subset_type = agent"
##  [29] "^SUBSET = GDS3309_2"
##  [30] "!subset_dataset_id = GDS3309"
##  [31] "!subset_description = cigarette smoke"
##  [32] "!subset_sample_id = GSM227869,GSM227872,GSM227873,GSM227875,GSM227879,GSM22788
1,GSM227882"
##  [33] "!subset_type = agent"
##  [34] "^Annotation"
##  [35] "!Annotation_date = Aug 09 2016"
##  [36] "!Annotation_platform = GPL571"
##  [37] "!Annotation_platform_title = [HG-U133A_2] Affymetrix Human Genome U133A 2.0 Ar
ray"
##  [38] "!Annotation_platform_organism = Homo sapiens"
##  [39] "^DATASET = GDS3309"
##  [40] "#ID_REF = Platform reference identifier"
##  [41] "#IDENTIFIER = identifier"
##  [42] "#GSM227868 = Value for GSM227868: Nose10 (Never Smoker); src: Nasal epithelial
samples from Never Smoker"
##  [43] "#GSM227870 = Value for GSM227870: Nose12 (Never Smoker); src: Nasal epithelial
samples from Never Smoker"
##  [44] "#GSM227871 = Value for GSM227871: Nose13 (Never Smoker); src: Nasal epithelial
```

```
samples from Never Smoker"
##  [45] "#GSM227874 = Value for GSM227874: Nose16 (Never Smoker); src: Nasal epithelial
samples from Never Smoker"
##  [46] "#GSM227876 = Value for GSM227876: Nose31 (Never Smoker); src: Nasal epithelial
samples from Never Smoker"
##  [47] "#GSM227877 = Value for GSM227877: Nose32 (Never Smoker); src: Nasal epithelial
samples from Never Smoker"
##  [48] "#GSM227878 = Value for GSM227878: Nose33 (Never Smoker); src: Nasal epithelial
samples from Never Smoker"
##  [49] "#GSM227880 = Value for GSM227880: Nose35 (Never Smoker); src: Nasal epithelial
samples from Never Smoker"
##  [50] "#GSM227869 = Value for GSM227869: Nose11 (Current Smoker); src: Nasal epitheli
al samples from Current Smoker"
##  [51] "#GSM227872 = Value for GSM227872: Nose14 (Current Smoker); src: Nasal epitheli
al samples from Current Smoker"
##  [52] "#GSM227873 = Value for GSM227873: Nose15 (Current Smoker); src: Nasal epitheli
al samples from Current Smoker"
##  [53] "#GSM227875 = Value for GSM227875: Nose17 (Current Smoker); src: Nasal epitheli
al samples from Current Smoker"
##  [54] "#GSM227879 = Value for GSM227879: Nose34 (Current Smoker); src: Nasal epitheli
al samples from Current Smoker"
##  [55] "#GSM227881 = Value for GSM227881: Nose36 (Current Smoker); src: Nasal epitheli
al samples from Current Smoker"
##  [56] "#GSM227882 = Value for GSM227882: Nose37 (Current Smoker); src: Nasal epitheli
al samples from Current Smoker"
##  [57] "#Gene title = Entrez Gene name"
##  [58] "#Gene symbol = Entrez Gene symbol"
##  [59] "#Gene ID = Entrez Gene identifier"
##  [60] "#UniGene title = Entrez UniGene name"
##  [61] "#UniGene symbol = Entrez UniGene symbol"
##  [62] "#UniGene ID = Entrez UniGene identifier"
##  [63] "#Nucleotide Title = Entrez Nucleotide title"
##  [64] "#GI = GenBank identifier"
##  [65] "#GenBank Accession = GenBank accession"
##  [66] "#Platform_CLONEID = CLONE_ID from Platform data table"
##  [67] "#Platform_ORF = ORF from Platform data table"
##  [68] "#Platform_SPOTID = SPOT_ID from Platform data table"
##  [69] "#Chromosome location = Entrez gene chromosome and location"
##  [70] "#Chromosome annotation = Entrez gene chromosome annotation"
##  [71] "#GO:Function = Gene Ontology Function term"
##  [72] "#GO:Process = Gene Ontology Process term"
##  [73] "#GO:Component = Gene Ontology Component term"
##  [74] "#GO:Function ID = Gene Ontology Function identifier"
##  [75] "#GO:Process ID = Gene Ontology Process identifier"
##  [76] "#GO:Component ID = Gene Ontology Component identifier"
##  [77] "!dataset_table_begin"
##  [78] "ID_REF\tIDENTIFIER\tGSM227868\tGSM227870\tGSM227871\tGSM227874\tGSM227876\tGSM
227877\tGSM227878\tGSM227880\tGSM227869\tGSM227872\tGSM227873\tGSM227875\tGSM227879\tGSM
227881\tGSM227882\tGene title\tGene symbol\tGene ID\tUniGene title\tUniGene symbol\tUniG
ene ID\tNucleotide Title\tGI\tGenBank Accession\tPlatform_CLONEID\tPlatform_ORF\tPlatfor
m_SPOTID\tChromosome location\tChromosome annotation\tGO:Function\tGO:Process\tGO:Compon
ent\tGO:Function ID\tGO:Process ID\tGO:Component ID"
```

```
start_line <- grep("!dataset_table_begin", raw_data)
my_data <- read.delim(file_path, skip = start_line, header = TRUE)

expression_data <- my_data %>% select(starts_with("GSM"))

identifier_column <- my_data["IDENTIFIER"]


# Normalize the data
normalized_data <- normalizeBetweenArrays(as.matrix(expression_data))

expression_df <- cbind(identifier_column, expression_data)

# Sample GSM code status mapping as a vector
gsm_status <- c(
  "GSM227868" = "Never Smoker",
  "GSM227870" = "Never Smoker",
  "GSM227871" = "Never Smoker",
  "GSM227874" = "Never Smoker",
  "GSM227876" = "Never Smoker",
  "GSM227877" = "Never Smoker",
  "GSM227878" = "Never Smoker",
  "GSM227880" = "Never Smoker",
  "GSM227869" = "Current Smoker",
  "GSM227872" = "Current Smoker",
  "GSM227873" = "Current Smoker",
  "GSM227875" = "Current Smoker",
  "GSM227879" = "Current Smoker",
  "GSM227881" = "Current Smoker",
  "GSM227882" = "Current Smoker"
)

# Ensure that the GSM codes in expression_df match the order of gsm_status
gsm_codes <- colnames(expression_df) # Exclude the 'IDENTIFIER' column

# Create a new row of smoker status matching the GSM codes
smoker_status <- sapply(gsm_codes, function(gsm) gsm_status[gsm])

# Add the smoker status as a new row to the dataframe
expression_df_with_status <- rbind(smoker_status, expression_df)


# Reshaping data for plotting purposes
long_df <- expression_df %>%
    pivot_longer(cols = starts_with("GSM"),
                 names_to = "Sample",
                 values_to = "Expression")

#Decided to take top 25 genes for better visualization purposes
#Steps to take top 25 genes:
#One, Calculate the mean expression for each gene
top_genes <- long_df %>%
```

```
    group_by(IDENTIFIER) %>%
    summarize(MeanExpression = mean(Expression, na.rm = TRUE)) %>%
    top_n(25, MeanExpression) %>%
    pull(IDENTIFIER)

#Two, Filter the long dataframe to include only the top genes
filtered_long_df <- long_df %>%
    filter(IDENTIFIER %in% top_genes)

#Three,plot with filtered data
#Scatter plot with geom_point

ggplot(filtered_long_df, aes(x = IDENTIFIER, y = Expression, color = Sample)) +
  geom_point() +
  scale_x_discrete(expand = expansion(mult = c(0.001, 0.01))) +  # Adjust the space betw
een labels
  theme(axis.text.x = element_text(angle = 90,
                                   vjust = 0.5,
                                   hjust = 1,
                                   margin = margin(t = 15))) +
  labs(title = "Top 25 Genes by Expression Across Samples",
       x = "Gene Names",
       y = "Expression Levels") +
  coord_flip() + # Optionally flip the coordinates
  theme_minimal()
```



```
#Alternative visualization: Table
#I've commented this out as I prefer the scatter plot
#top_25_genes <- filtered_long_df %>%
#  select(IDENTIFIER, Sample, Expression) %>%
#  arrange(IDENTIFIER, Sample)
#print(top_25_genes)
#save to CSV
#write.csv(top_25_genes_table, "top_25_genes_expression_table.csv", row.names = FALSE)



#Principle Component Analysis

# Scale the expression data
expression_data_scaled <- scale(expression_data)

# Replace infinite values with 0
expression_data_scaled[is.infinite(expression_data_scaled)] <- 0

# Replace missing (NA) values with 0
expression_data_scaled[is.na(expression_data_scaled)] <- 0

# Check the dimensions of the scaled data to ensure it's still valid
cat("Dimensions of the scaled expression data:", dim(expression_data_scaled), "\n")
```

```
## Dimensions of the scaled expression data: 22278 15
```

```
# Perform PCA
pca_result <- prcomp(expression_data_scaled, center = TRUE, scale. = TRUE)

# Print PCA summary to check the result
summary(pca_result)
```

```
## Importance of components:
##                           PC1     PC2    PC3     PC4    PC5     PC6     PC7
## Standard deviation     3.7379 0.67714 0.3529 0.33913 0.2511 0.22946 0.22106
## Proportion of Variance 0.9315 0.03057 0.0083 0.00767 0.0042 0.00351 0.00326
## Cumulative Proportion  0.9315 0.96202 0.9703 0.97799 0.9822 0.98570 0.98896
##                            PC8     PC9    PC10    PC11    PC12   PC13    PC14
## Standard deviation     0.20233 0.18110 0.15753 0.14637 0.12975 0.1098 0.09324
## Proportion of Variance 0.00273 0.00219 0.00165 0.00143 0.00112 0.0008 0.00058
## Cumulative Proportion  0.99169 0.99387 0.99553 0.99696 0.99808 0.9989 0.99946
##                           PC15
## Standard deviation     0.08979
## Proportion of Variance 0.00054
## Cumulative Proportion  1.00000
```

```
# Step 4: Extract PCA Results and Add Metadata
# Create a data frame with PCA results
pca_df <- as.data.frame(pca_result$x)



colnames(pca_df)[1:15] <- colnames(expression_data)


# Remove the 'Sample' column (which is the 16th column in this case)
#pca_df <- pca_df %>% select(-Sample)


gsm_samples <- colnames(pca_df)[1:15]

# Create a new row corresponding to SmokerStatus using the gsm_status mapping
smoker_status_row <- gsm_status[gsm_samples]

# Combine the SmokerStatus row with the existing pca_df
# We use rbind to add the SmokerStatus row to the top of the dataframe
pca_df <- rbind(SmokerStatus = smoker_status_row, pca_df)

#pca_df <- pca_df %>% slice(-22280)
# Check the result to ensure SmokerStatus is added correctly at the top
#print(pca_df)




# Assuming pca_df already contains your PCA results with SmokerStatus as the first row

# Extract the PCA coordinates (PC1, PC2, etc.) and SmokerStatus
# The SmokerStatus is the first row, so let's separate it from the rest of the PCA data
smoker_status <- pca_df[1, 1:15]  # Extract the SmokerStatus row (first row)
pca_data <- pca_df[-1, 1:15]  # Remove the SmokerStatus row to keep only PCA values

# Convert smoker_status into a factor to categorize it as "Never Smoker" or "Current Smo
ker"
# Extract the SmokerStatus as a vector (first row)
smoker_status <- as.vector(as.matrix(pca_df[1, 1:15]))  # Convert first row to a vector

# Convert smoker_status into a factor
smoker_status <- as.factor(smoker_status)

# Remove the SmokerStatus row from pca_df to keep only PCA data for plotting
pca_data <- pca_df[-1, 1:15]

# Convert PCA data into numeric for plotting
pca_data <- as.data.frame(lapply(pca_data, as.numeric))

# Transpose the PCA data so that samples are in rows and PCs in columns
pca_plot_df <- as.data.frame(t(pca_data))
```

```
# Add SmokerStatus as a column in the transposed PCA dataframe
pca_plot_df$SmokerStatus <- smoker_status

# Plot PCA with ggplot2, using PC1 and PC2, and color points by SmokerStatus
ggplot(pca_plot_df, aes(x = V1, y = V2, color = SmokerStatus)) +
  geom_point(size = 3) +
  labs(title = "PCA of Gene Expression Data: PC1 vs PC2",
       x = "Principal Component 1",
       y = "Principal Component 2") +
  theme_minimal() +
  scale_color_manual(values = c("Never Smoker" = "blue", "Current Smoker" = "red"))
```