Extraccction Insc by flyccellatlas
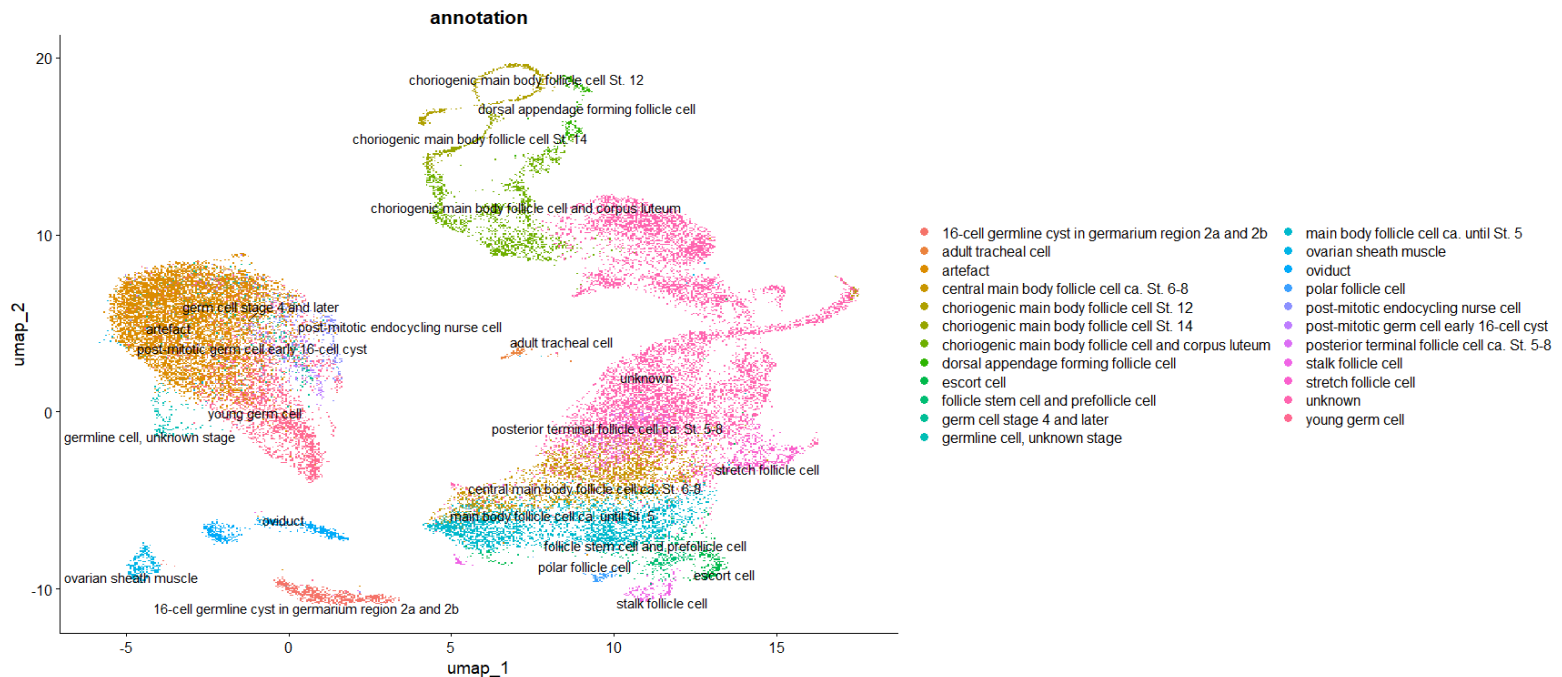
#load library

Hide

```
library(Seurat)
library(ggplot2)
library(ggrepel)
library(SeuratDisk)
```

Load data

Identify cells expressing "insc" > 1

Hide

```
insc_expression <- adata@assays@RNA@data["insc", ]
annotation_cells <- colnames(adata)[adata$annotation == "16-cell germline cyst in germarium region 2a and 2b"]
insc_positive <- insc_expression[annotation_cells] > 1
```

Hide

```
# Create two subsets: insc+ and insc-
with_insc <- annotation_cells[insc_positive]
without_insc <- annotation_cells[!insc_positive]
```

Hide

```
# Extract the expression matrix
group1_expression <- adata@assays$RNA@data[, with_insc]
group2_expression <- adata@assays$RNA@data[, without_insc]
```

```
# List of genes
all_genes <- rownames(adata@assays$RNA@data)

# Filter genes based on their expression
mean_expression_with_insc <- rowMeans(group1_expression)
mean_expression_without_insc <- rowMeans(group2_expression)
```

```
# Keep only those genes that have an average expression greater than 1 in at least one of the groups
genes_to_keep <- (mean_expression_with_insc > 1) | (mean_expression_without_insc > 1)

# Filter expression matrices and gene list
group1_expression <- group1_expression[genes_to_keep, ]
group2_expression <- group2_expression[genes_to_keep, ]
all_genes <- all_genes[genes_to_keep]


# Calculate the fold change and p-values
results <- data.frame(Gene = all_genes, logFC = numeric(length(all_genes)), p.value = numeric(length(all_ge
nes)))
```

T-test code

```
for (gene in all_genes) {
  t_test_result <- t.test(group1_expression[gene, ], group2_expression[gene, ])
  fold_change <- mean(group2_expression[gene, ]) / mean(group1_expression[gene, ])
  log2_fold_change <- log2(fold_change)
  results[results$Gene == gene, "logFC"] <- log2_fold_change
  results[results$Gene == gene, "p.value"] <- t_test_result$p.value
}

# Adjust p-values
results$adj.p.value <- p.adjust(results$p.value, method = "BH")


results$annotation <- ifelse(abs(results$logFC) > 1 & results$adj.p.value < 0.05,
                             ifelse(results$logFC > 0, "without_insc",
                                    "with_insc"),
                             "Not significant")
```

#Graphic

```r
# Volcano Plot Chart
p <- ggplot(results, aes(x = logFC, y = -log10(adj.p.value), color = annotation)) +
  geom_point(alpha = 0.6, size = 2) +
  scale_color_manual(values = c("with_insc" = "blue",
                                "without_insc" = "red",
                                "Not significant" = "black")) +
  theme_minimal() +
  labs(title = "Volcano Plot of Differential Gene Expression",
       subtitle = "Data source: Ovary, 16-cell germline cyst in germarium region 2a and 2b",#here you chang
e the name of the annotation you use
    x = "Log2 Fold Change",
    y = "-Log10 Adjusted P-Value",
    caption = "Analysis method: t-test"
  ) +
  geom_hline(yintercept = -log10(0.05), color = "grey", linetype = "dashed") +
  geom_vline(xintercept = c(-1, 1), color = "grey", linetype = "dashed") +
  geom_text_repel(data = subset(results, abs(logFC) > 1 & -log10(adj.p.value) > 2),
                  aes(label = Gene), size = 3) +
  theme(
    text = element_text(family = "Arial", size = 12),
    legend.position = "top",
    legend.title = element_blank()
  )

print(p)
```

Volcano Plot of Differential Gene Expression

Data source: Ovary, 16-cell germline cyst in germarium region 2a and 2b

Legend: Not significant · with_insc · without_insc

- X-axis: Log2 Fold Change
- Y-axis: -Log10 Adjusted P-Value

Labeled genes: insc, lok, pgc, Prip, osk, swa, tos, Hs3st-A, lncRNA:CR45667, CG32719, CG42260, eag, futsch, disco-r, CG15628, fred

Analysis method: t-test
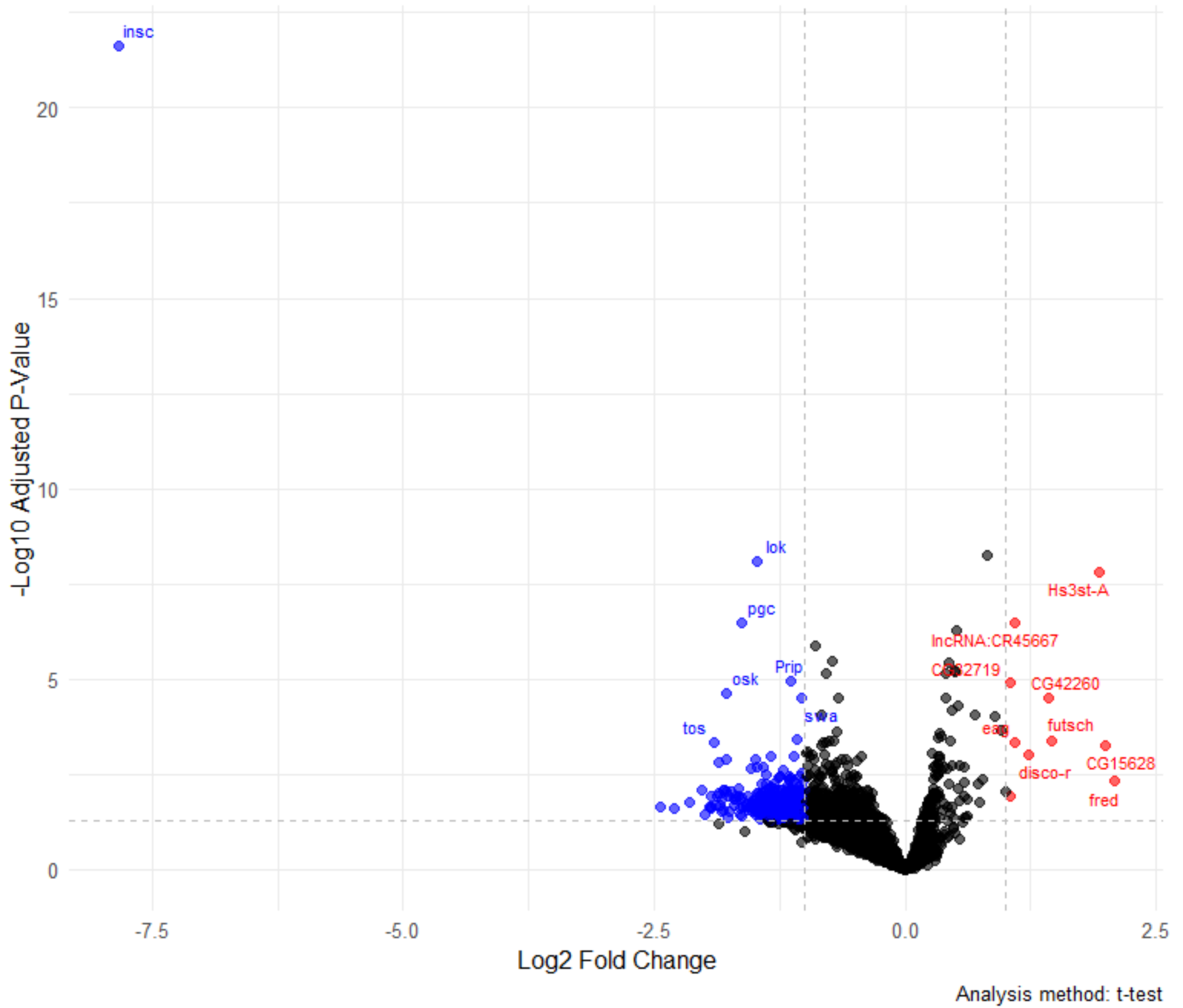
Hide

```
session_info()
```

```
Warning: running command '"quarto" TMPDIR=C:/Users/omcn8/AppData/Local/Temp/RtmpUrGeCK/file6cec1a153236 -V'
had status 1
```

```
─ Session info ──────────────────────────────────────────────────────────────────
 setting  value
 version  R version 4.4.3 (2025-02-28 ucrt)
 os       Windows 11 x64 (build 26100)
 system   x86_64, mingw32
 ui       RStudio
 language (EN)
 collate  English_United States.utf8
 ctype    English_United States.utf8
 tz       America/Chicago
 date     2025-04-11
 rstudio  2024.12.1+563 Kousa Dogwood (desktop)
 pandoc   3.2 @ C:/Program Files/RStudio/resources/app/bin/quarto/bin/tools/ (via rmarkdown)
 quarto   ERROR: Unknown command "TMPDIR=C:/Users/omcn8/AppData/Local/Temp/RtmpUrGeCK/file6cec1a153236". Di
d you mean command "preview"? @ C:\\PROGRA~1\\RStudio\\RESOUR~1\\app\\bin\\quarto\\bin\\quarto.exe

─ Packages ──────────────────────────────────────────────────────────────────────
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 abind                1.4-8     2024-09-12 [1] CRAN (R 4.4.1)
 AnnotationDbi        1.68.0    2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
 Biobase              2.66.0    2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
 BiocFileCache        2.14.0    2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
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 biomaRt            * 2.62.1    2025-01-30 [1] Bioconductor 3.20 (R 4.4.2)
 Biostrings           2.74.1    2024-12-16 [1] Bioconductor 3.20 (R 4.4.2)
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 bit64                4.6.0-1   2025-01-16 [1] CRAN (R 4.4.3)
 blob                 1.2.4     2023-03-17 [1] CRAN (R 4.4.3)
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 callr                3.7.6     2024-03-25 [1] CRAN (R 4.4.3)
 cli                  3.6.4     2025-02-13 [1] CRAN (R 4.4.3)
 cluster              2.1.8     2024-12-11 [2] CRAN (R 4.4.3)
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 colorspace           2.1-1     2024-07-26 [1] CRAN (R 4.4.3)
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 devtools           * 2.4.5     2022-10-11 [1] CRAN (R 4.4.3)
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future                1.34.0      2024-07-29 [1] CRAN (R 4.4.3)
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ggrepel             * 0.9.6       2024-09-07 [1] CRAN (R 4.4.3)
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glue                  1.8.0       2024-09-30 [1] CRAN (R 4.4.3)
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grr                   0.9.5       2016-08-26 [1] CRAN (R 4.4.0)
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ica                   1.0-3       2022-07-08 [1] CRAN (R 4.4.0)
igraph                2.1.4       2025-01-23 [1] CRAN (R 4.4.3)
IRanges               2.40.1      2024-12-05 [1] Bioconductor 3.20 (R 4.4.2)
irlba                 2.3.5.1     2022-10-03 [1] CRAN (R 4.4.3)
jquerylib             0.1.4       2021-04-26 [1] CRAN (R 4.4.3)
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KernSmooth            2.23-26     2025-01-01 [2] CRAN (R 4.4.3)
knitr                 1.50        2025-03-16 [1] CRAN (R 4.4.3)
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lmtest                0.9-40      2022-03-21 [1] CRAN (R 4.4.3)
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Matrix              * 1.7-2       2025-01-23 [2] CRAN (R 4.4.3)
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munsell               0.5.1       2024-04-01 [1] CRAN (R 4.4.3)
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parallelly            1.43.0      2025-03-24 [1] CRAN (R 4.4.3)
patchwork           * 1.3.0       2024-09-16 [1] CRAN (R 4.4.3)
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pkgload           1.4.0        2024-06-28 [1] CRAN (R 4.4.3)
plotly            4.10.4       2024-01-13 [1] CRAN (R 4.4.3)
plyr              1.8.9        2023-10-02 [1] CRAN (R 4.4.3)
png               0.1-8        2022-11-29 [1] CRAN (R 4.4.0)
polyclip          1.10-7       2024-07-23 [1] CRAN (R 4.4.1)
prettyunits       1.2.0        2023-09-24 [1] CRAN (R 4.4.3)
processx          3.8.6        2025-02-21 [1] CRAN (R 4.4.3)
profvis           0.4.0        2024-09-20 [1] CRAN (R 4.4.3)
progress          1.2.3        2023-12-06 [1] CRAN (R 4.4.3)
progressr         0.15.1       2024-11-22 [1] CRAN (R 4.4.3)
promises          1.3.2        2024-11-28 [1] CRAN (R 4.4.3)
ps                1.9.0        2025-02-18 [1] CRAN (R 4.4.3)
purrr             1.0.4        2025-02-05 [1] CRAN (R 4.4.3)
R.methodsS3       1.8.2        2022-06-13 [1] CRAN (R 4.4.0)
R.oo              1.27.0       2024-11-01 [1] CRAN (R 4.4.1)
R.utils           2.13.0       2025-02-24 [1] CRAN (R 4.4.3)
R6                2.6.1        2025-02-15 [1] CRAN (R 4.4.3)
RANN              2.6.2        2024-08-25 [1] CRAN (R 4.4.3)
rappdirs          0.3.3        2021-01-31 [1] CRAN (R 4.4.3)
RColorBrewer      1.1-3        2022-04-03 [1] CRAN (R 4.4.0)
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remotes           2.5.0        2024-03-17 [1] CRAN (R 4.4.3)
reshape2          1.4.4        2020-04-09 [1] CRAN (R 4.4.3)
reticulate        1.42.0       2025-03-25 [1] CRAN (R 4.4.3)
rlang             1.1.5        2025-01-17 [1] CRAN (R 4.4.3)
rmarkdown         2.29         2024-11-04 [1] CRAN (R 4.4.3)
ROCR              1.0-11       2020-05-02 [1] CRAN (R 4.4.3)
RSpectra          0.16-2       2024-07-18 [1] CRAN (R 4.4.3)
RSQLite           2.3.9        2024-12-03 [1] CRAN (R 4.4.3)
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Rtsne             0.17         2023-12-07 [1] CRAN (R 4.4.3)
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S4Vectors         0.44.0       2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
sass              0.4.9        2024-03-15 [1] CRAN (R 4.4.3)
scales            1.3.0        2023-11-28 [1] CRAN (R 4.4.3)
scattermore       1.2          2023-06-12 [1] CRAN (R 4.4.3)
sctransform     * 0.4.1        2023-10-19 [1] CRAN (R 4.4.3)
sessioninfo       1.2.3        2025-02-05 [1] CRAN (R 4.4.3)
Seurat          * 5.2.1        2025-01-24 [1] CRAN (R 4.4.3)
SeuratDisk      * 0.0.0.9021   2025-04-11 [1] Github (mojaveazure/seurat-disk@877d4e1)
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shiny             1.10.0       2024-12-14 [1] CRAN (R 4.4.3)
sp              * 2.2-0        2025-02-01 [1] CRAN (R 4.4.3)
spam              2.11-1       2025-01-20 [1] CRAN (R 4.4.3)
SparseArray       1.6.2        2025-02-20 [1] Bioconductor 3.20 (R 4.4.2)
sparseMatrixStats 1.18.0       2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
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spatstat.explore  3.4-2        2025-03-21 [1] CRAN (R 4.4.3)
spatstat.geom     3.3-6        2025-03-18 [1] CRAN (R 4.4.3)
spatstat.random   3.3-3        2025-03-19 [1] CRAN (R 4.4.3)
spatstat.sparse   3.1-0        2024-06-21 [1] CRAN (R 4.4.3)
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stringi                1.8.7       2025-03-27 [1] CRAN (R 4.4.3)
stringr                1.5.1       2023-11-14 [1] CRAN (R 4.4.3)
SummarizedExperiment   1.36.0      2024-10-29 [1] Bioconductor 3.20 (R 4.4.1)
survival               3.8-3       2024-12-17 [2] CRAN (R 4.4.3)
tensor                 1.5         2012-05-05 [1] CRAN (R 4.4.0)
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tidyr                  1.3.1       2024-01-24 [1] CRAN (R 4.4.3)
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uwot                   0.2.3       2025-02-24 [1] CRAN (R 4.4.3)
vctrs                  0.6.5       2023-12-01 [1] CRAN (R 4.4.3)
viridis              * 0.6.1       2021-05-11 [1] CRAN (R 4.4.3)
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zoo                    1.8-13      2025-02-22 [1] CRAN (R 4.4.3)

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[2] C:/Program Files/R/R-4.4.3/library
* — Packages attached to the search path.
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