Intro_to_R_for_biologists

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Load library

```
library(tidyverse)
```

```
## -- Attaching packages ------------------------------------------------ tidyverse 1.3.0 --

## v ggplot2 3.3.2     v purrr   0.3.4
## v tibble  3.0.2     v dplyr   1.0.0
## v tidyr   1.1.0     v stringr 1.4.0
## v readr   1.3.1     v forcats 0.5.0

## -- Conflicts --------------------------------------------------- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

Read in breast cancer RNA-seq data

```
counts = read_csv("GSE60450_GeneLevel_Normalized(CPM.and.TMM)_data.csv")
```

```
## Warning: Missing column names filled in: 'X1' [1]

## Parsed with column specification:
## cols(
##   X1 = col_character(),
##   gene_symbol = col_character(),
##   GSM1480291 = col_double(),
##   GSM1480292 = col_double(),
##   GSM1480293 = col_double(),
##   GSM1480294 = col_double(),
##   GSM1480295 = col_double(),
##   GSM1480296 = col_double(),
##   GSM1480297 = col_double(),
##   GSM1480298 = col_double(),
##   GSM1480299 = col_double(),
##   GSM1480300 = col_double(),
##   GSM1480301 = col_double(),
##   GSM1480302 = col_double()
## )
```

```
sampleInfo = read_csv("GSE60450_filtered_metadata.csv")
```

```
## Warning: Missing column names filled in: 'X1' [1]
```

```
## Parsed with column specification:
## cols(
##   X1 = col_character(),
##   characteristics = col_character(),
##   immunophenotype = col_character(),
##   `developmental stage` = col_character()
## )
```

view what is stored in variables

```
sampleInfo
```

```
## Warning: `...` is not empty.
##
## We detected these problematic arguments:
## * `needs_dots`
##
## These dots only exist to allow future extensions and should be empty.
## Did you misspecify an argument?
```

```
## # A tibble: 12 x 4
##    X1       characteristics                immunophenotype    `developmental st~
##    <chr>    <chr>                          <chr>              <chr>
##  1 GSM1480~ mammary gland, luminal cells,~ luminal cell popu~ virgin
##  2 GSM1480~ mammary gland, luminal cells,~ luminal cell popu~ virgin
##  3 GSM1480~ mammary gland, luminal cells,~ luminal cell popu~ 18.5 day pregnancy
##  4 GSM1480~ mammary gland, luminal cells,~ luminal cell popu~ 18.5 day pregnancy
##  5 GSM1480~ mammary gland, luminal cells,~ luminal cell popu~ 2 day lactation
##  6 GSM1480~ mammary gland, luminal cells,~ luminal cell popu~ 2 day lactation
##  7 GSM1480~ mammary gland, basal cells, v~ basal cell popula~ virgin
##  8 GSM1480~ mammary gland, basal cells, v~ basal cell popula~ virgin
##  9 GSM1480~ mammary gland, basal cells, 1~ basal cell popula~ 18.5 day pregnancy
## 10 GSM1480~ mammary gland, basal cells, 1~ basal cell popula~ 18.5 day pregnancy
## 11 GSM1480~ mammary gland, basal cells, 2~ basal cell popula~ 2 day lactation
## 12 GSM1480~ mammary gland, basal cells, 2~ basal cell popula~ 2 day lactation
```

```
counts
```

```
## Warning: `...` is not empty.
##
## We detected these problematic arguments:
## * `needs_dots`
##
## These dots only exist to allow future extensions and should be empty.
## Did you misspecify an argument?
```

```
## # A tibble: 23,735 x 14
##    X1    gene_symbol GSM1480291 GSM1480292 GSM1480293 GSM1480294 GSM1480295
##    <chr> <chr>            <dbl>      <dbl>      <dbl>      <dbl>      <dbl>
##  1 ENSM~ Gnai3          243.      256.         240.       217.       84.7
##  2 ENSM~ Pbsn             0         0            0          0         0
##  3 ENSM~ Cdc45           11.2      13.8         11.6        4.27      8.35
##  4 ENSM~ H19              6.31      8.53         7.09      11.0       0.194
##  5 ENSM~ Scml2            2.19      4.66         2.80       2.50      1.24
##  6 ENSM~ Apoh             0.224     0.0840       0          0         0
##  7 ENSM~ Narf            11.3      14.7         26.2       18.8      14.7
##  8 ENSM~ Cav2           118.      113.          50.5       63.4     186.
##  9 ENSM~ Klf6          2036.     2230.        1903.      1960.     1094.
## 10 ENSM~ Scmh1           33.7      38.7          9.18       9.43      3.92
## # ... with 23,725 more rows, and 7 more variables: GSM1480296 <dbl>,
## #   GSM1480297 <dbl>, GSM1480298 <dbl>, GSM1480299 <dbl>, GSM1480300 <dbl>,
## #   GSM1480301 <dbl>, GSM1480302 <dbl>
```

dimension of variables-> rows by columns

```
dim(sampleInfo)
```

```
## [1] 12  4
```

```
dim(counts)
```

```
## [1] 23735    14
```

view the first 6 lines by default or specify more lines through Arg

```
head(sampleInfo)
```

```
## Warning: `...` is not empty.
##
## We detected these problematic arguments:
## * `needs_dots`
##
## These dots only exist to allow future extensions and should be empty.
## Did you misspecify an argument?
```

```
## # A tibble: 6 x 4
##   X1       characteristics                immunophenotype    `developmental st~
##   <chr>    <chr>                          <chr>              <chr>
## 1 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ virgin
## 2 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ virgin
## 3 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 18.5 day pregnancy
## 4 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 18.5 day pregnancy
## 5 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 2 day lactation
## 6 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 2 day lactation
```

```
?head # check Arg n
```

```
## starting httpd help server ... done
```

```
head(sampleInfo, 8)
```

```
## Warning: `...` is not empty.
##
## We detected these problematic arguments:
## * `needs_dots`
##
## These dots only exist to allow future extensions and should be empty.
## Did you misspecify an argument?
```

```
## # A tibble: 8 x 4
##   X1       characteristics                 immunophenotype    `developmental st~
##   <chr>    <chr>                           <chr>              <chr>
## 1 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ virgin
## 2 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ virgin
## 3 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 18.5 day pregnancy
## 4 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 18.5 day pregnancy
## 5 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 2 day lactation
## 6 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 2 day lactation
## 7 GSM1480~ mammary gland, basal cells, vi~ basal cell popula~ virgin
## 8 GSM1480~ mammary gland, basal cells, vi~ basal cell popula~ virgin
```

view the last 6 lines

```
tail(sampleInfo)
```

```
## Warning: `...` is not empty.
##
## We detected these problematic arguments:
## * `needs_dots`
##
## These dots only exist to allow future extensions and should be empty.
## Did you misspecify an argument?
```

```
## # A tibble: 6 x 4
##   X1       characteristics                 immunophenotype    `developmental st~
##   <chr>    <chr>                           <chr>              <chr>
## 1 GSM1480~ mammary gland, basal cells, vi~ basal cell popula~ virgin
## 2 GSM1480~ mammary gland, basal cells, vi~ basal cell popula~ virgin
## 3 GSM1480~ mammary gland, basal cells, 18~ basal cell popula~ 18.5 day pregnancy
## 4 GSM1480~ mammary gland, basal cells, 18~ basal cell popula~ 18.5 day pregnancy
## 5 GSM1480~ mammary gland, basal cells, 2 ~ basal cell popula~ 2 day lactation
## 6 GSM1480~ mammary gland, basal cells, 2 ~ basal cell popula~ 2 day lactation
```

view the whole variable

```
View(sampleInfo)# or just click on the variable in the Environment pane
View(counts)
```

view column vectors

```
sampleInfo$X1
```

```
##  [1] "GSM1480291" "GSM1480292" "GSM1480293" "GSM1480294" "GSM1480295"
##  [6] "GSM1480296" "GSM1480297" "GSM1480298" "GSM1480299" "GSM1480300"
## [11] "GSM1480301" "GSM1480302"
```

```
sampleInfo$immunophenotype
```

```
##  [1] "luminal cell population" "luminal cell population"
##  [3] "luminal cell population" "luminal cell population"
##  [5] "luminal cell population" "luminal cell population"
##  [7] "basal cell population"   "basal cell population"
##  [9] "basal cell population"   "basal cell population"
## [11] "basal cell population"   "basal cell population"
```

view values from a to b [a:b] in a column vector

```
sampleInfo$X1[1:3]
```

```
## [1] "GSM1480291" "GSM1480292" "GSM1480293"
```

```
sampleInfo$X1[2:4]
```

```
## [1] "GSM1480292" "GSM1480293" "GSM1480294"
```

```
sampleInfo$immunophenotype[1:3]
```

```
## [1] "luminal cell population" "luminal cell population"
## [3] "luminal cell population"
```

view the structure of the data

```
str(sampleInfo)
```

```
## tibble [12 x 4] (S3: spec_tbl_df/tbl_df/tbl/data.frame)
##  $ X1                 : chr [1:12] "GSM1480291" "GSM1480292" "GSM1480293" "GSM1480294" ...
##  $ characteristics    : chr [1:12] "mammary gland, luminal cells, virgin" "mammary gland, luminal cel
##  $ immunophenotype    : chr [1:12] "luminal cell population" "luminal cell population" "luminal cell
##  $ developmental stage: chr [1:12] "virgin" "virgin" "18.5 day pregnancy" "18.5 day pregnancy" ...
##  - attr(*, "spec")=
##   .. cols(
##   ..   X1 = col_character(),
##   ..   characteristics = col_character(),
##   ..   immunophenotype = col_character(),
##   ..   `developmental stage` = col_character()
##   .. )
```

```
str(counts)
```

```
## tibble [23,735 x 14] (S3: spec_tbl_df/tbl_df/tbl/data.frame)
##  $ X1         : chr [1:23735] "ENSMUSG00000000001" "ENSMUSG00000000003" "ENSMUSG00000000028" "ENSMUS
##  $ gene_symbol: chr [1:23735] "Gnai3" "Pbsn" "Cdc45" "H19" ...
##  $ GSM1480291 : num [1:23735] 243.29 0 11.18 6.31 2.19 ...
##  $ GSM1480292 : num [1:23735] 255.66 0 13.78 8.53 4.66 ...
##  $ GSM1480293 : num [1:23735] 239.74 0 11.6 7.09 2.8 ...
##  $ GSM1480294 : num [1:23735] 217.1 0 4.27 11.04 2.5 ...
##  $ GSM1480295 : num [1:23735] 84.744 0 8.35 0.194 1.243 ...
##  $ GSM1480296 : num [1:23735] 84.599 0 8.199 0 0.855 ...
##  $ GSM1480297 : num [1:23735] 175.04 0 12.11 2.12 5.79 ...
##  $ GSM1480298 : num [1:23735] 187.49 0 11.1 1.19 8.8 ...
##  $ GSM1480299 : num [1:23735] 176.66 0 7.53 1.55 9.81 ...
##  $ GSM1480300 : num [1:23735] 169.094 0 7.099 0.867 7.47 ...
##  $ GSM1480301 : num [1:23735] 158.45 0 1.98 10.83 7.57 ...
##  $ GSM1480302 : num [1:23735] 133.59 0 2.88 5.77 9.88 ...
##  - attr(*, "spec")=
##   .. cols(
##   ..   X1 = col_character(),
##   ..   gene_symbol = col_character(),
##   ..   GSM1480291 = col_double(),
##   ..   GSM1480292 = col_double(),
##   ..   GSM1480293 = col_double(),
##   ..   GSM1480294 = col_double(),
##   ..   GSM1480295 = col_double(),
##   ..   GSM1480296 = col_double(),
##   ..   GSM1480297 = col_double(),
##   ..   GSM1480298 = col_double(),
##   ..   GSM1480299 = col_double(),
##   ..   GSM1480300 = col_double(),
##   ..   GSM1480301 = col_double(),
##   ..   GSM1480302 = col_double()
##   .. )
```

summary of data: length of string vectors refers to num of coordinates, whereas for numerical vectors: min, max, 1st quartile, 2nd quartile(median), 3rd quartile

```
summary(counts)
```

```
##       X1             gene_symbol          GSM1480291          GSM1480292
##  Length:23735       Length:23735       Min.   :    0.000   Min.   :    0.000
##  Class :character   Class :character   1st Qu.:    0.000   1st Qu.:    0.000
##  Mode  :character   Mode  :character   Median :    1.745   Median :    1.891
##                                        Mean   :   42.132   Mean   :   42.132
##                                        3rd Qu.:   29.840   3rd Qu.:   29.604
##                                        Max.   :12525.066   Max.   :12416.211
##    GSM1480293         GSM1480294         GSM1480295          GSM1480296
##  Min.   :    0.00   Min.   :    0.00   Min.   :     0.00   Min.   :     0.00
##  1st Qu.:    0.00   1st Qu.:    0.00   1st Qu.:     0.00   1st Qu.:     0.00
##  Median :    0.92   Median :    0.89   Median :     0.58   Median :     0.54
##  Mean   :   42.13   Mean   :   42.13   Mean   :    42.13   Mean   :    42.13
```

```
##  3rd Qu.:   21.91   3rd Qu.:   19.92   3rd Qu.:    12.27   3rd Qu.:    12.28
##  Max.   :49191.15   Max.   :55692.09   Max.   :111850.87   Max.   :108726.08
##    GSM1480297          GSM1480298          GSM1480299
##  Min.   :    0.000   Min.   :    0.000   Min.   :    0.000
##  1st Qu.:    0.000   1st Qu.:    0.000   1st Qu.:    0.000
##  Median :    2.158   Median :    2.254   Median :    1.854
##  Mean   :   42.132   Mean   :   42.132   Mean   :   42.132
##  3rd Qu.:   27.414   3rd Qu.:   26.450   3rd Qu.:   24.860
##  Max.   :10489.311   Max.   :10662.486   Max.   :15194.048
##    GSM1480300          GSM1480301          GSM1480302
##  Min.   :    0.000   Min.   :    0.000   Min.   :    0.000
##  1st Qu.:    0.000   1st Qu.:    0.000   1st Qu.:    0.000
##  Median :    1.816   Median :    1.629   Median :    1.749
##  Mean   :   42.132   Mean   :   42.132   Mean   :   42.132
##  3rd Qu.:   23.443   3rd Qu.:   23.443   3rd Qu.:   24.818
##  Max.   :17434.935   Max.   :19152.728   Max.   :15997.193
```

```
summary(sampleInfo)
```

```
##       X1            characteristics    immunophenotype    developmental stage
##  Length:12          Length:12          Length:12          Length:12
##  Class :character   Class :character   Class :character   Class :character
##  Mode  :character   Mode  :character   Mode  :character   Mode  :character
```

Excercises 1-4

```
# 1.
?head
head(sampleInfo, n = 8)
```

```
## Warning: '...' is not empty.
##
## We detected these problematic arguments:
## * 'needs_dots'
##
## These dots only exist to allow future extensions and should be empty.
## Did you misspecify an argument?
```

```
## # A tibble: 8 x 4
##   X1       characteristics                 immunophenotype    'developmental st~
##   <chr>    <chr>                           <chr>              <chr>
## 1 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ virgin
## 2 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ virgin
## 3 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 18.5 day pregnancy
## 4 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 18.5 day pregnancy
## 5 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 2 day lactation
## 6 GSM1480~ mammary gland, luminal cells, ~ luminal cell popu~ 2 day lactation
## 7 GSM1480~ mammary gland, basal cells, vi~ basal cell popula~ virgin
## 8 GSM1480~ mammary gland, basal cells, vi~ basal cell popula~ virgin
```

```r
# 2.
subsetCounts = head(counts, n = 20)
# 3.
subsetCounts$GSM1480291
```

```
##  [1]  243.28596    0.00000   11.18453    6.30808    2.19217    0.22369
##  [7]   11.27401  118.24288 2036.16657   33.68781  126.92208    0.67107
## [13]    0.04474    0.00000    0.26843    0.00000    0.67107   17.31366
## [19]   73.54949   75.74166
```

```r
# 4.
mean(subsetCounts$GSM1480291)
```

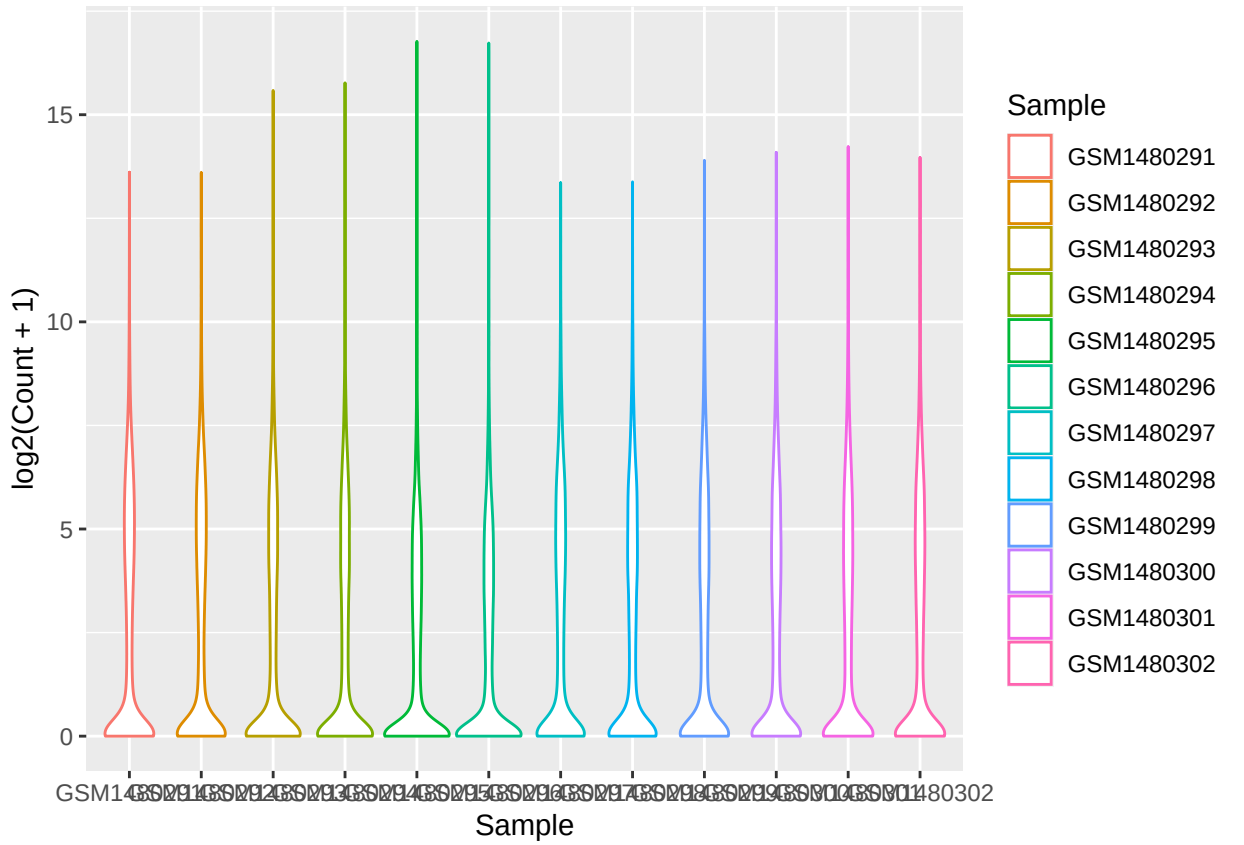
```
## [1] 137.8874
```

Formatting the data

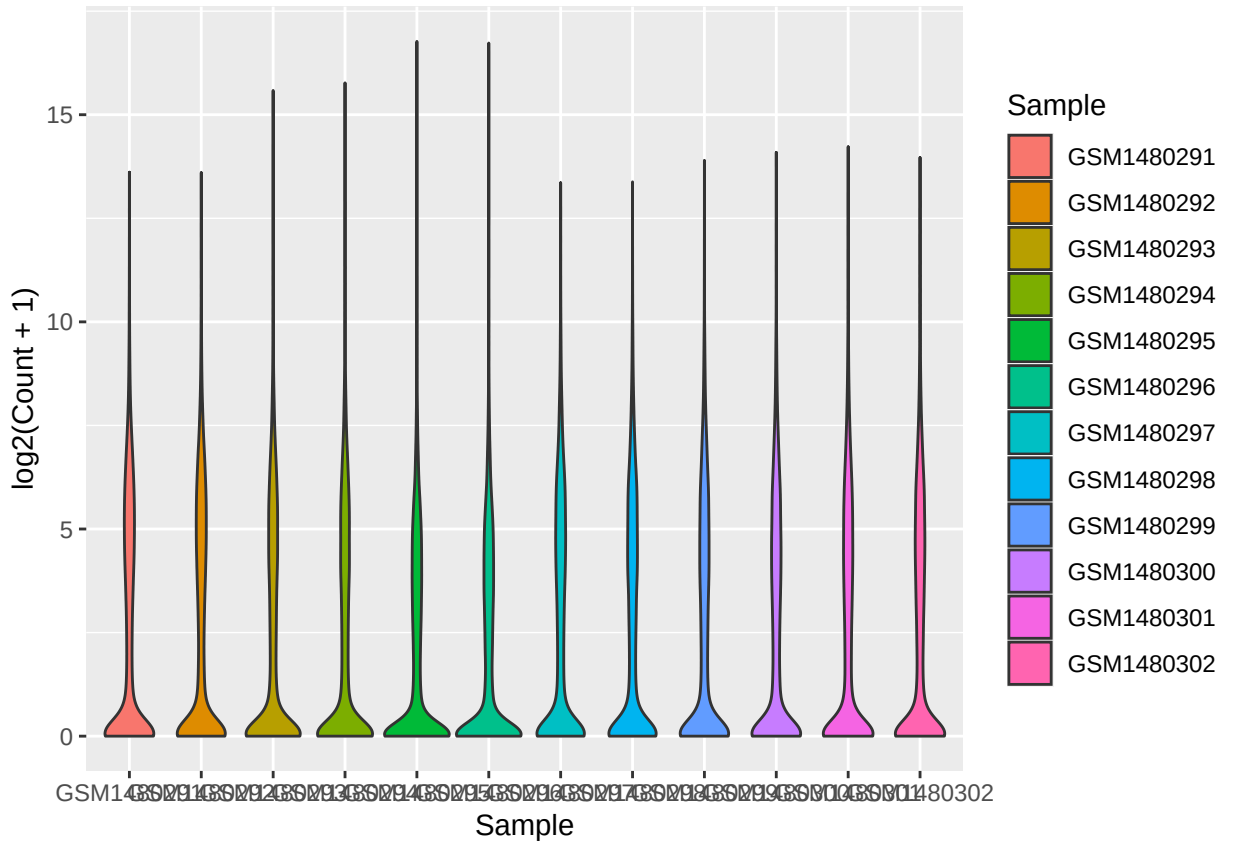
```r
seqData = pivot_longer(counts, col = starts_with('GSM'), names_to = 'Sample', values_to = 'Count')

# or
seqData = pivot_longer(counts, col = GSM1480291:GSM1480302, names_to = 'Sample', values_to = 'Count')

# or
seqData = pivot_longer(counts, col = -c('X1', 'gene_symbol'), names_to = 'Sample', values_to = 'Count')

allInfo = full_join(seqData, sampleInfo, by = c('Sample' = 'X1'))
```

Plot data

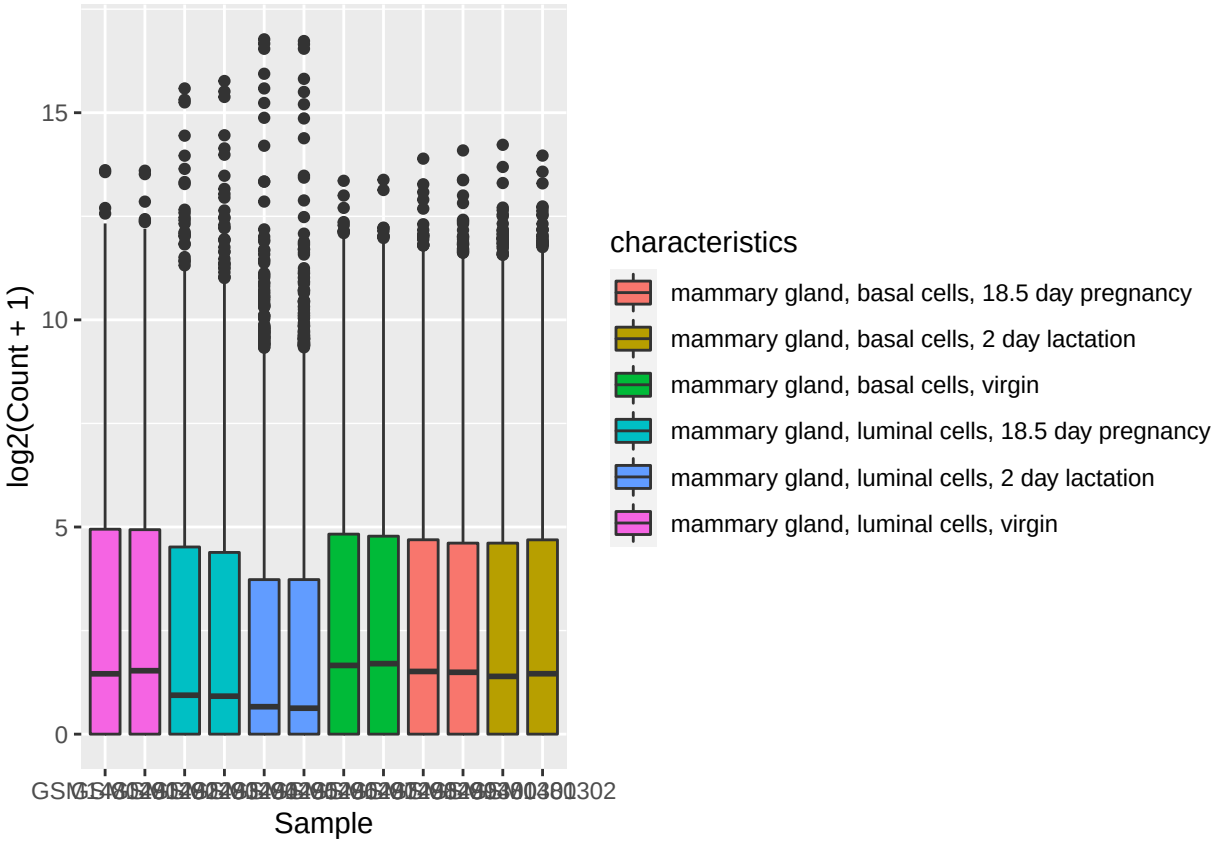
```r
ggplot(allInfo, mapping = aes(x = Sample, y = log2(Count + 1), colour = Sample)) +
  geom_violin()
```



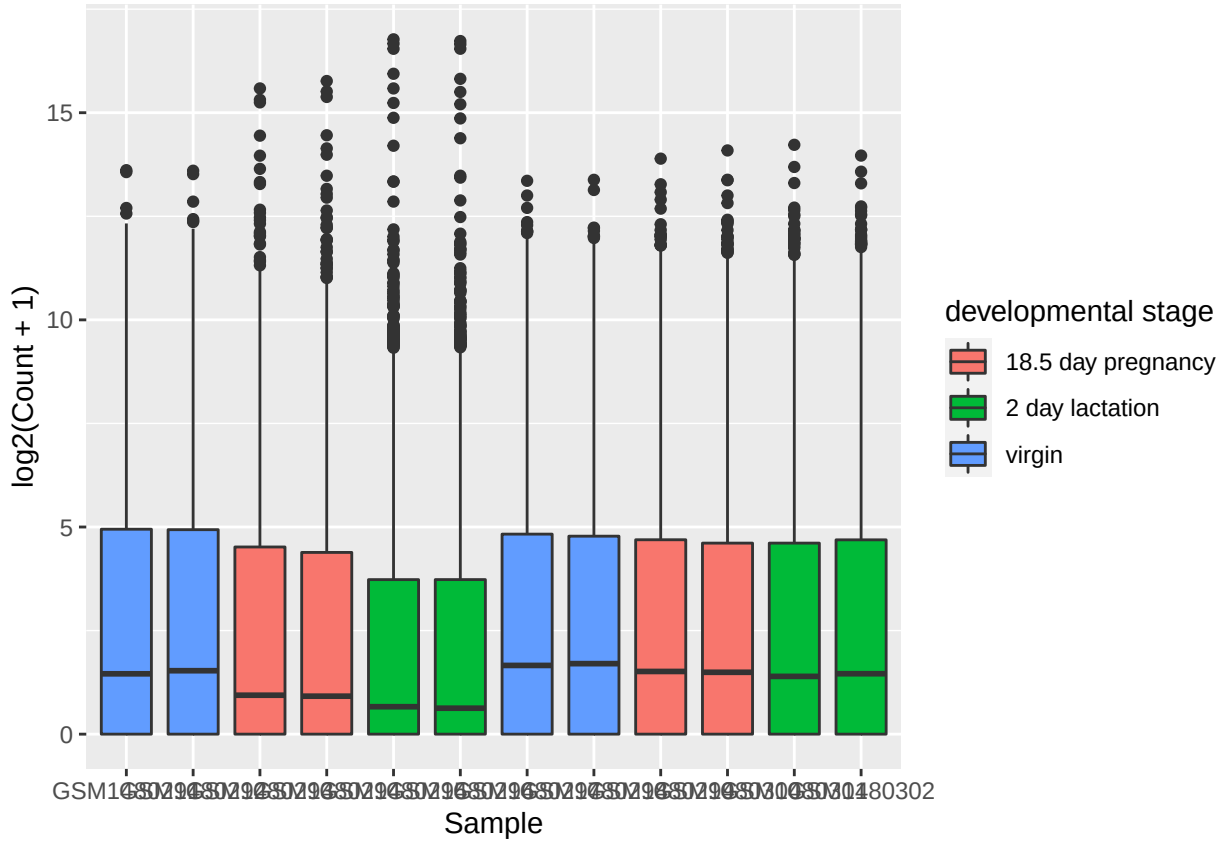

```
ggplot(allInfo, mapping = aes(x = Sample, y = log2(Count + 1), fill = Sample)) +
  geom_violin()
```




```
ggplot(allInfo, mapping = aes(x = Sample, y = log2(Count + 1),
                              fill = characteristics)) + geom_boxplot()
```




```
ggplot(allInfo, mapping = aes(x = Sample, y = log2(Count + 1),
                              fill = `developmental stage`)) + geom_boxplot()
```



Shorten Category names

```
allInfo <-  mutate(allInfo, Group = case_when(
  str_detect(characteristics, 'basal.*virgin') ~ 'bvirg',
  str_detect(characteristics, 'basal.*preg') ~ 'bpreg',
  str_detect(characteristics, 'basal.*lact') ~ 'blact',
  str_detect(characteristics, 'luminal.*virgin') ~ 'lvirg',
  str_detect(characteristics, 'luminal.*preg') ~ 'lpreg',
  str_detect(characteristics, 'luminal.*lact') ~ 'llact',
))
```

Select 8 genes with the highest counts summed across all samples

```
myGenes <-  allInfo %>%
  group_by(gene_symbol) %>%
  summarise(Total_count = sum(Count)) %>%  # remove repeated values
  arrange(desc(Total_count)) %>% # Arrange data into descending order
  head(n = 8) %>%
  pull(gene_symbol) # Pull out a single variable
```

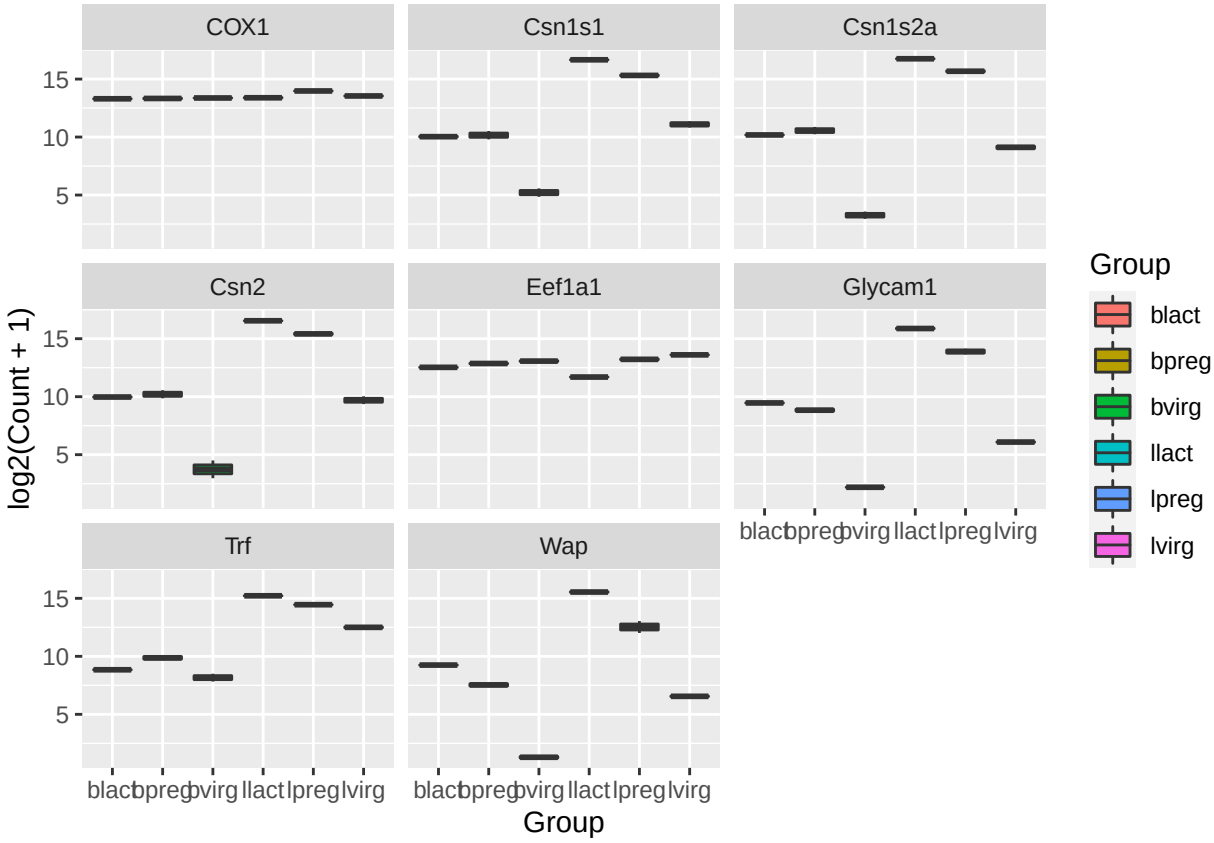
```
## `summarise()` ungrouping output (override with `.groups` argument)
```

Filter data

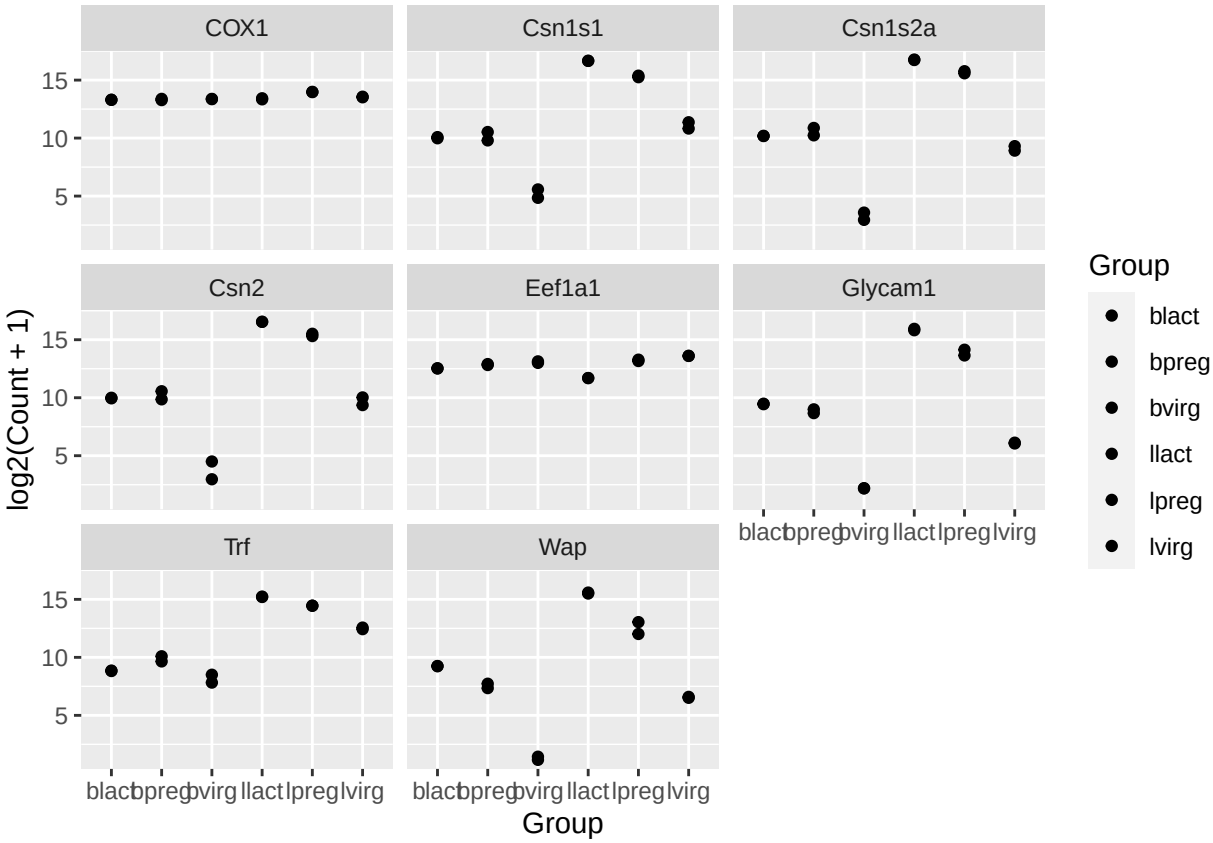
```
myGenesCounts = filter(allInfo, gene_symbol %in% myGenes)
```

Create plot for each of the 8 genes

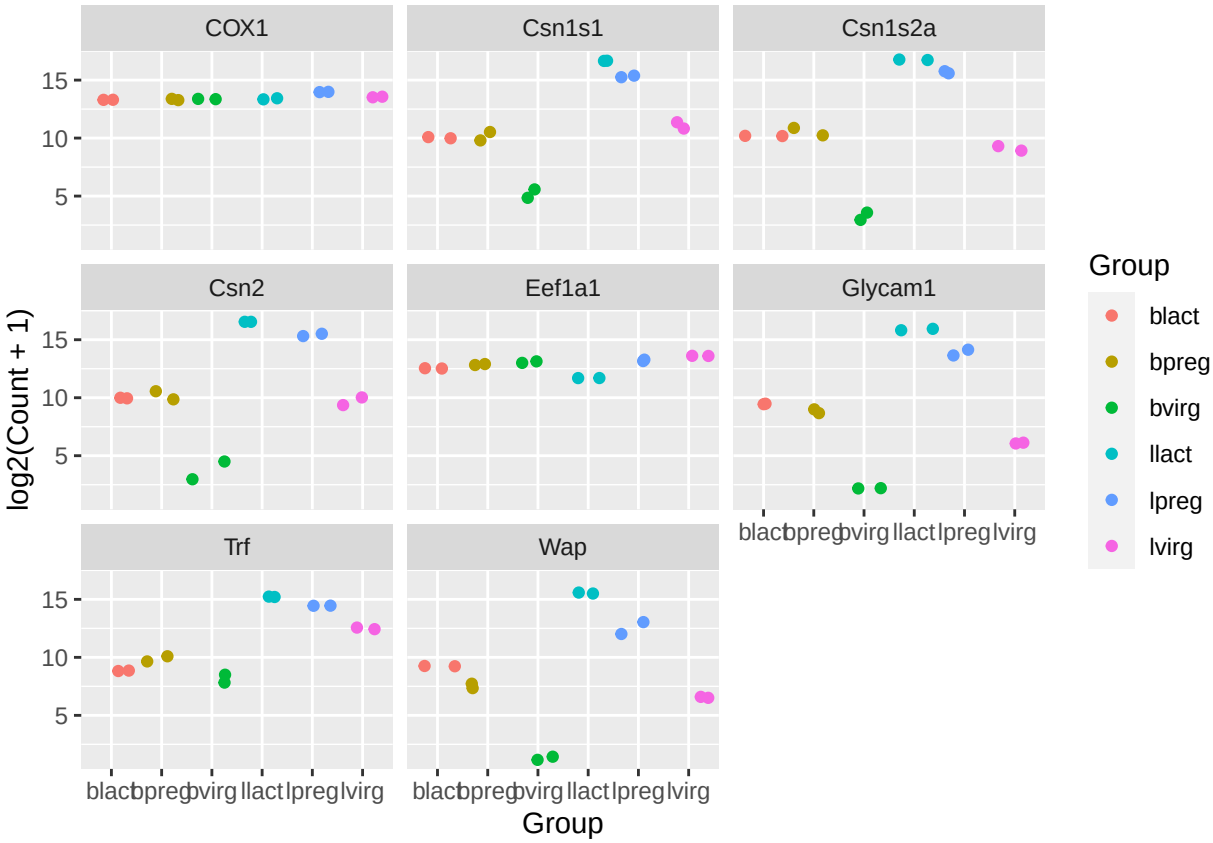
```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), fill = Group)) +
  geom_boxplot() +
  facet_wrap(~ gene_symbol)
```




```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1),fill = Group)) +
  geom_point() +
  facet_wrap(~ gene_symbol)
```

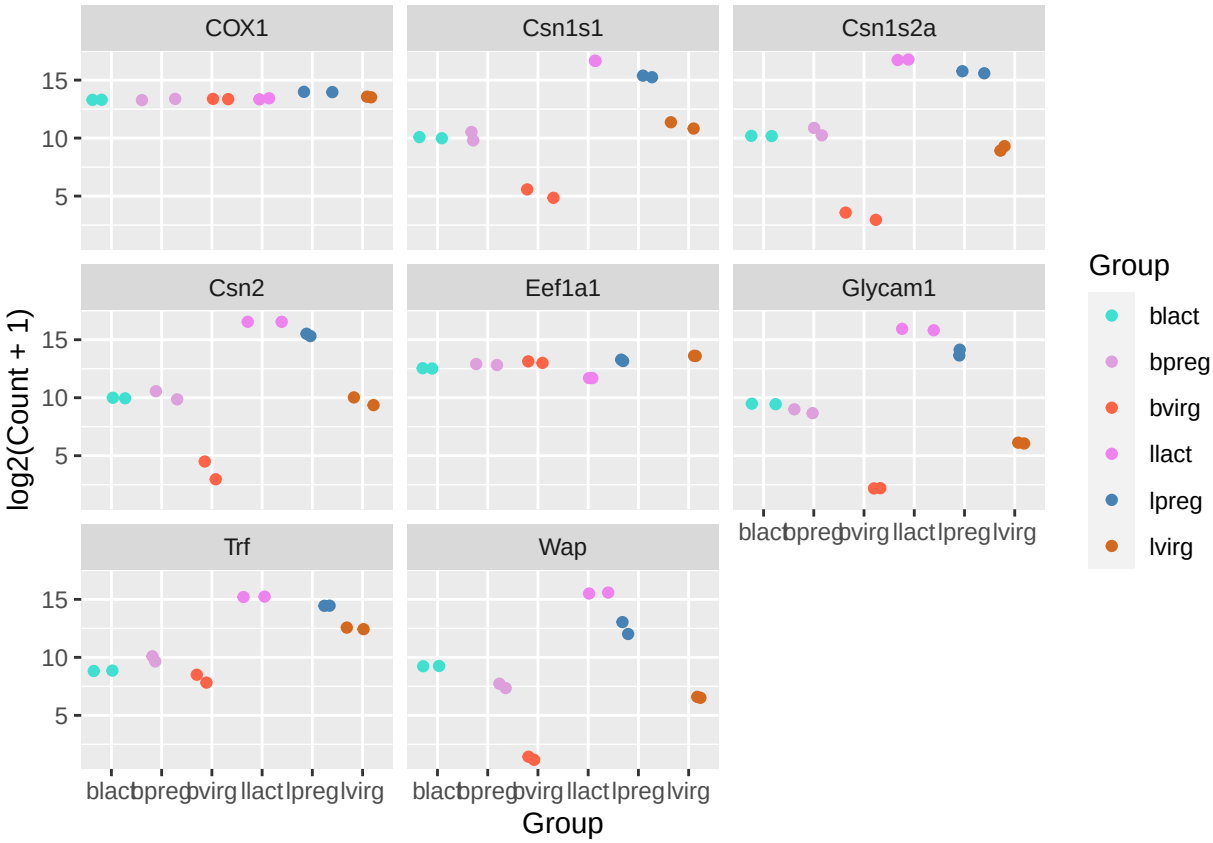



```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol)
```

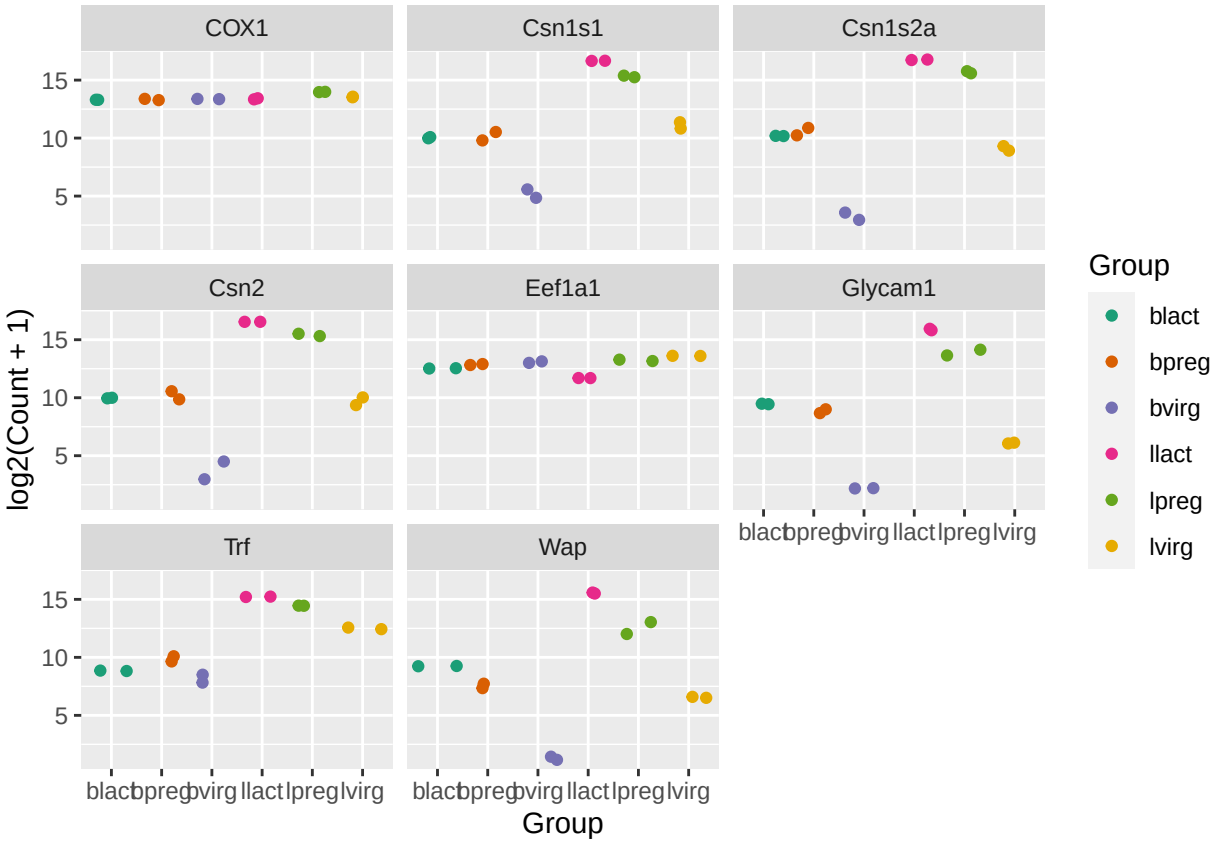


customise plots 1. colours

```
myColours = c('turquoise', 'plum', 'tomato', 'violet', 'steelblue', 'chocolate')

ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  scale_colour_manual(values = myColours)
```

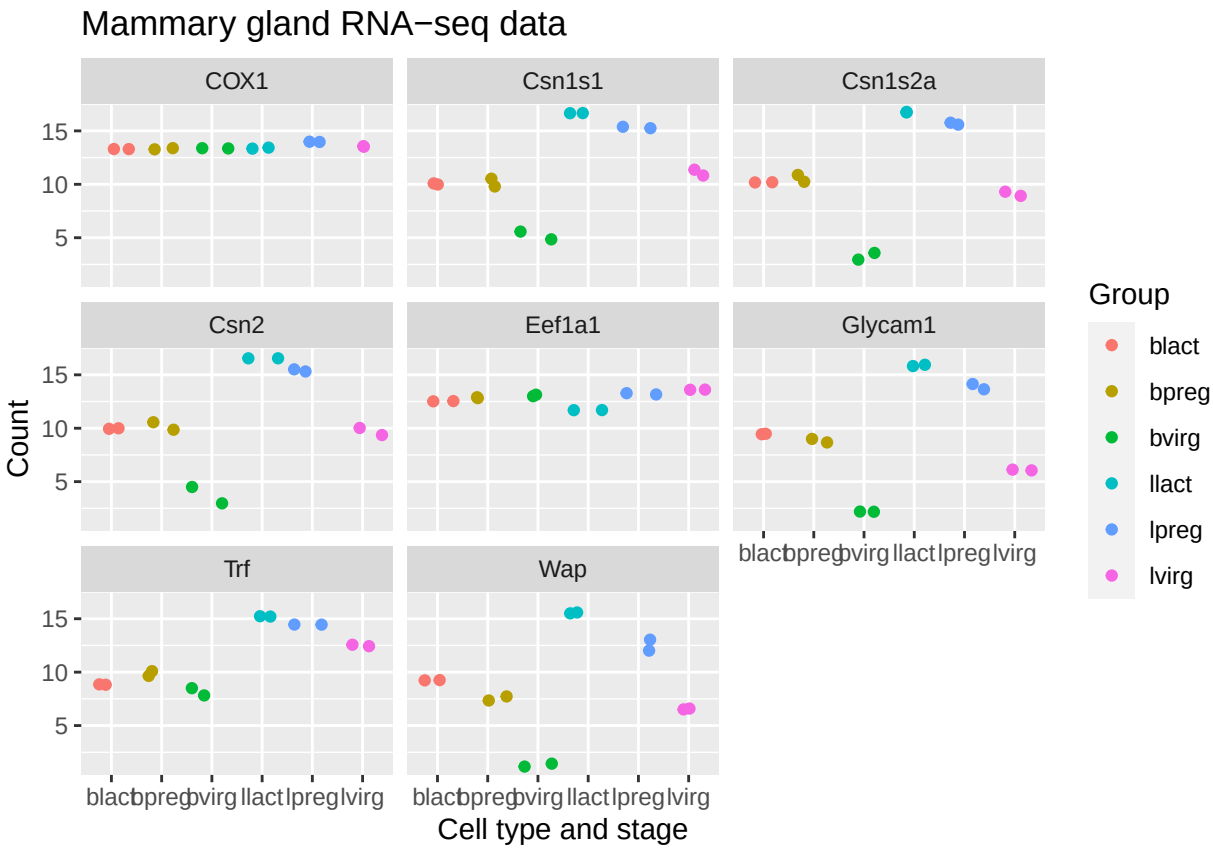



```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  scale_colour_brewer(palette = 'Dark2')
```



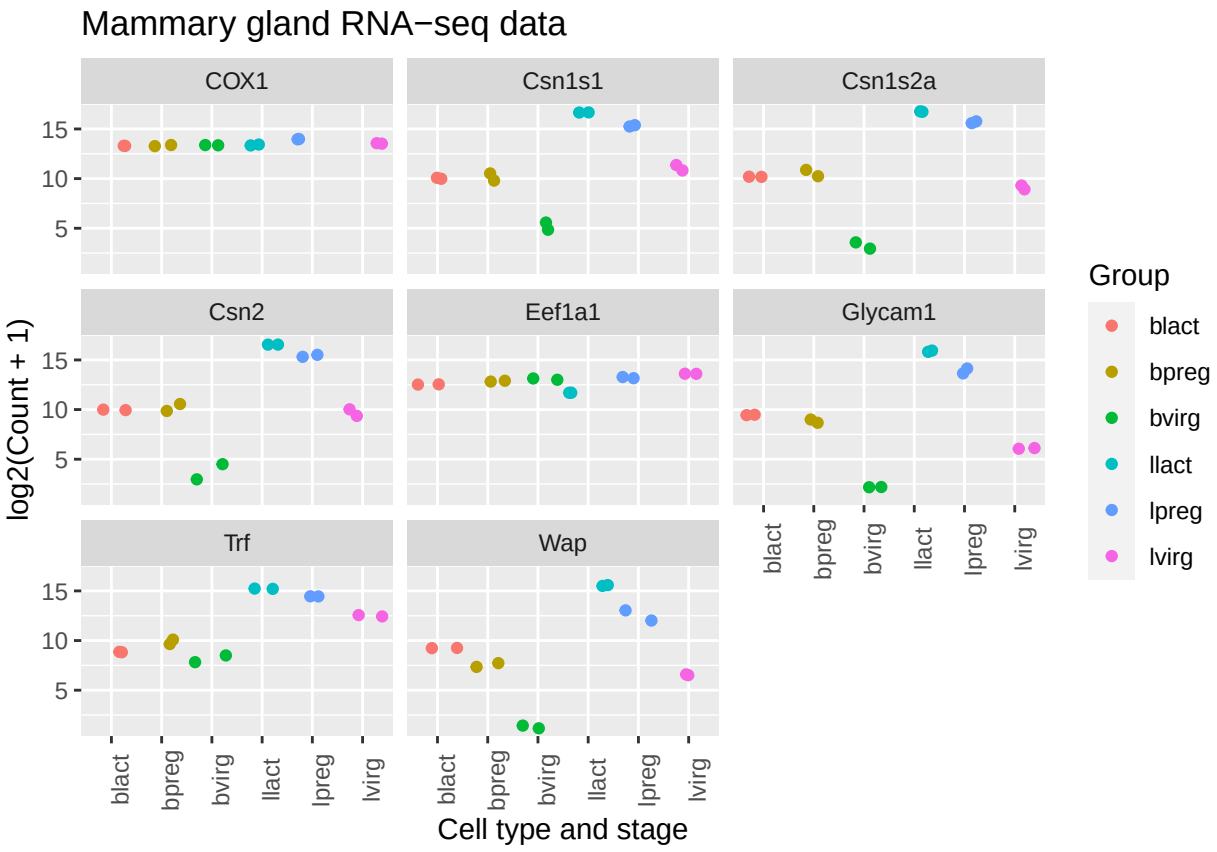
2. axis

```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  labs(x = 'Cell type and stage', y = 'Count',
       title = 'Mammary gland RNA-seq data')
```

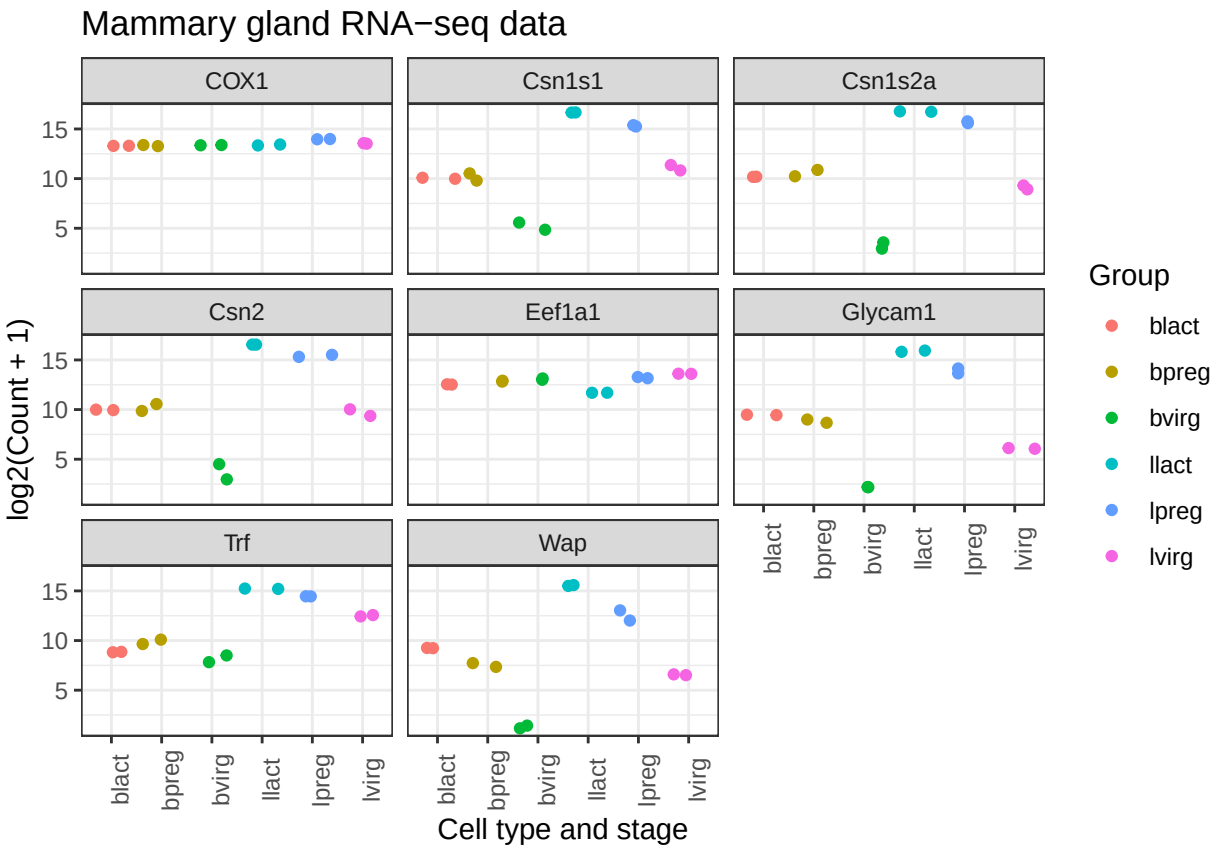


3. theme

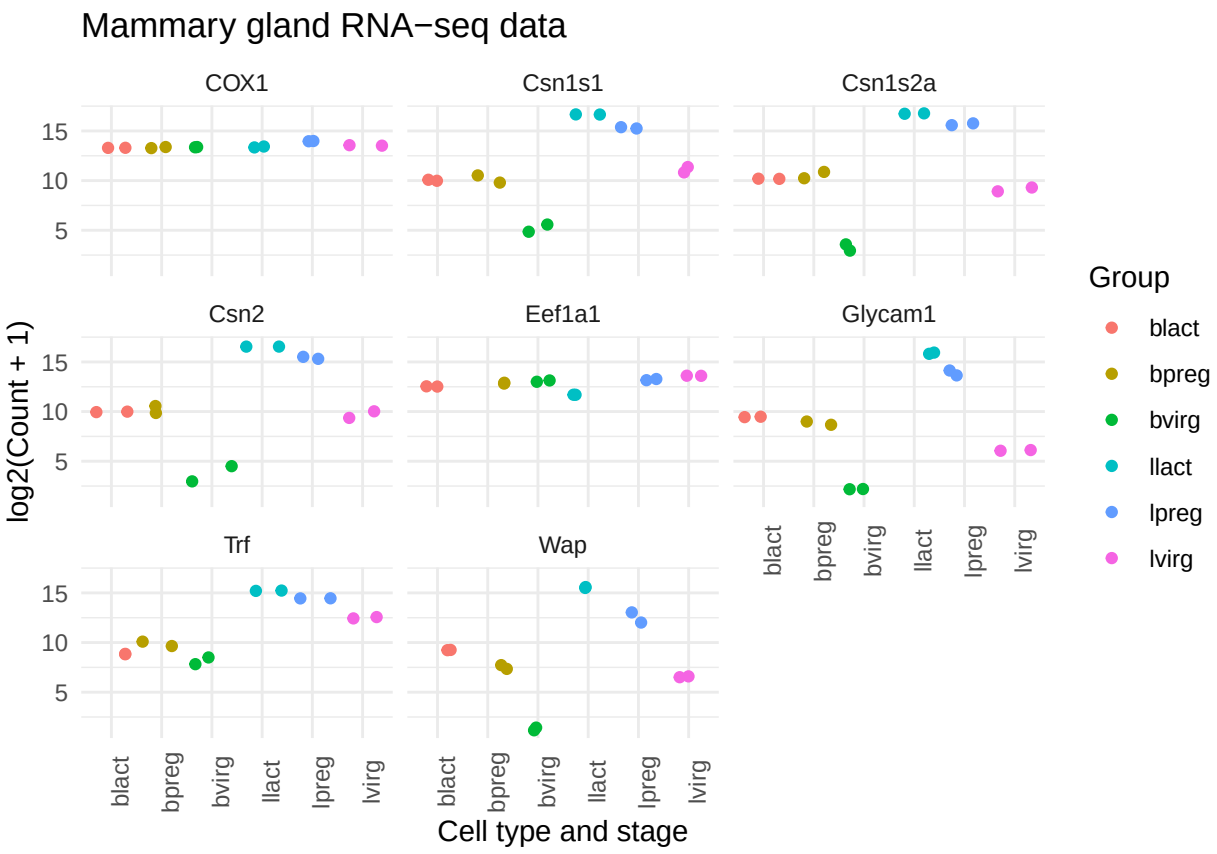
```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  labs(x = 'Cell type and stage', y = 'log2(Count + 1)',
       title = 'Mammary gland RNA-seq data') +
  theme(axis.text.x = element_text(angle = 90))
```



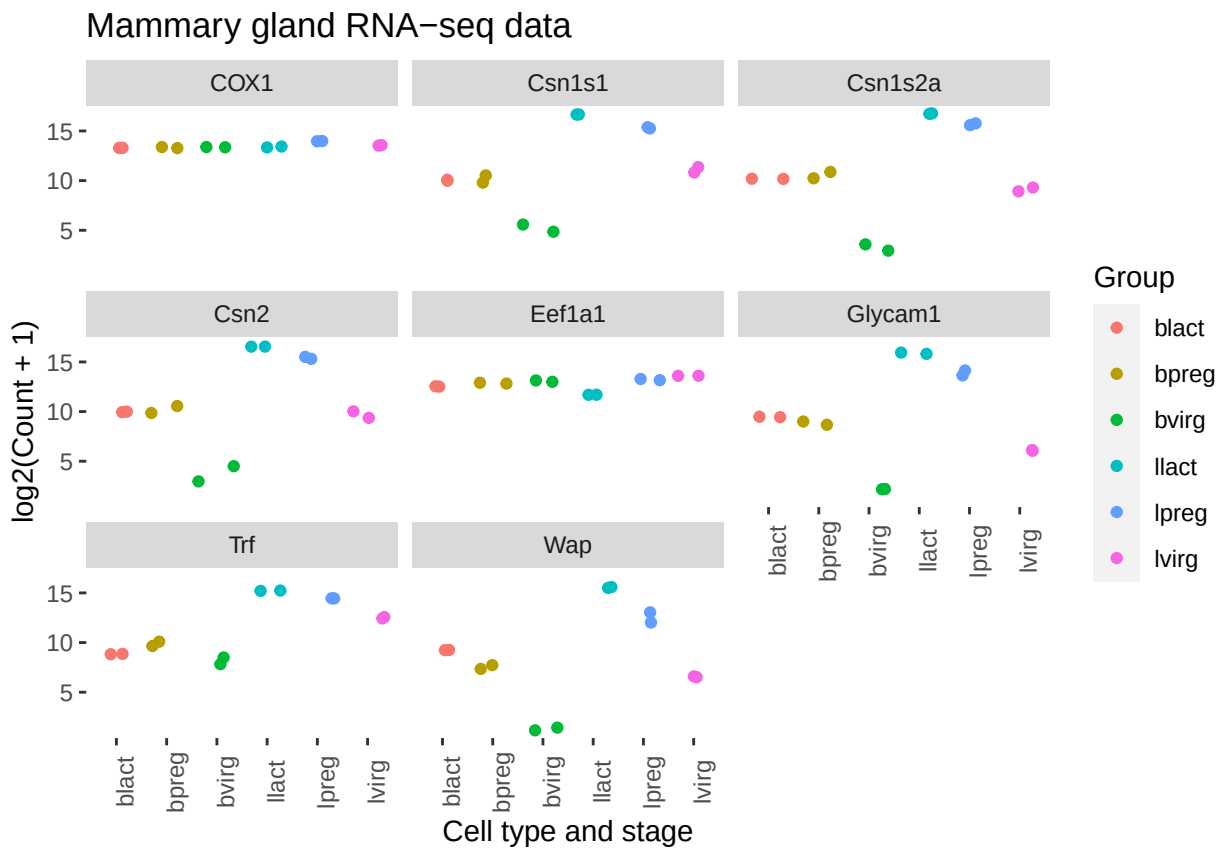

```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  labs(x = 'Cell type and stage', y = 'log2(Count + 1)',
       title = 'Mammary gland RNA-seq data') +
  theme_bw() +
  theme(axis.text.x = element_text(angle = 90))
```




```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  labs(x = 'Cell type and stage', y = 'log2(Count + 1)',
       title = 'Mammary gland RNA-seq data') +
  theme_minimal() +
  theme(axis.text.x = element_text(angle = 90))
```

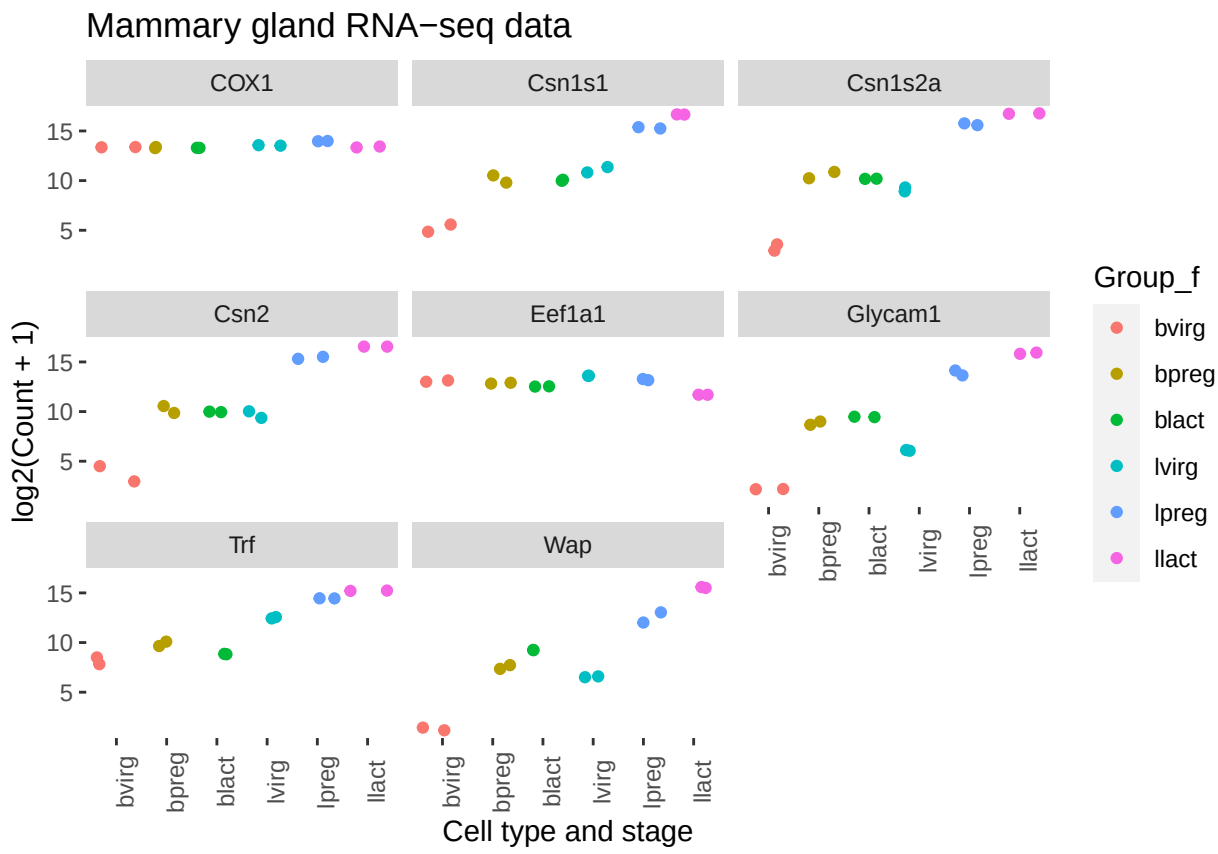



```
ggplot(data = myGenesCounts,
       mapping = aes(x = Group, y = log2(Count + 1), colour = Group)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  labs(x = 'Cell type and stage', y = 'log2(Count + 1)',
       title = 'Mammary gland RNA-seq data') +
  theme(axis.text.x = element_text(angle = 90)) +
  theme(panel.background = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank())
```



order and categories(levels)

```
groupOrder = c('bvirg', 'bpreg', 'blact', 'lvirg', 'lpreg', 'llact')

myGenesCounts = mutate(myGenesCounts, Group_f = factor(Group, levels = groupOrder))

ggplot(myGenesCounts,
       mapping = aes(x = Group_f, y = log2(Count + 1), colour = Group_f)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  labs(x = 'Cell type and stage', y = 'log2(Count + 1)',
       title = 'Mammary gland RNA-seq data') +
  theme(axis.text.x = element_text(angle = 90)) +
  theme(panel.background = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank())
```



save the plot

```
pdf('myplot.pdf')

ggplot(myGenesCounts, mapping = aes(x = Group_f, y = log2(Count + 1), colour = Group_f)) +
  geom_jitter() +
  facet_wrap(~ gene_symbol) +
  labs(x = 'Cell type and stage', y = 'log2(Count + 1)', title = 'Mammary gland RNA-seq data') +
  theme(axis.text.x = element_text(angle = 90)) +
  theme(panel.background = element_blank(),
        panel.grid.major = element_blank(),
        panel.grid.minor = element_blank())

dev.off()
```

```
## pdf
##   2
```

The end