A (Very) Short Introduction to R on Smell Testing Data for Wet Lab Scientists

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Review of Lecture 1

Pre-processing data: import and inspect data

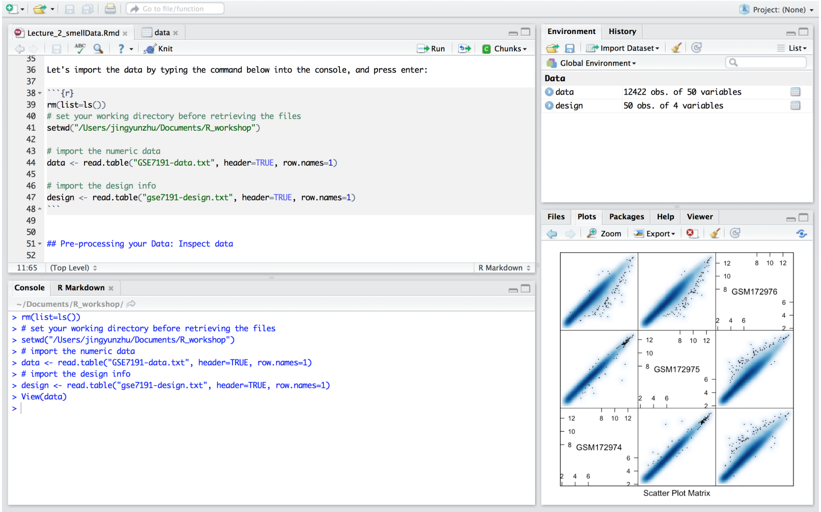
Plot your data: Stripplot, Scatter plot, Density plot, Box plot, Violin plot

Identify outlier sample via heatmap, pairwise correlation plot

Credits

Review of Lecture 1

Recapitulation of Rstudio features: layout and utility



~~~
Let's import the data by typing the command below into the console, and press enter:

```{r}
rm(list=ls())
# set your working directory before retrieving the files
setwd("/Users/jingyunzhu/Documents/R_workshop")

# import the numeric data
data <- read.table("GSE7191-data.txt", header=TRUE, row.names=1)

# import the design info
design <- read.table("gse7191-design.txt", header=TRUE, row.names=1)
```


## Pre-processing your Data: Inspect data
~~~

Data

data	12422 obs. of 50 variables
design	50 obs. of 4 variables

```
> rm(list=ls())
> # set your working directory before retrieving the files
> setwd("/Users/jingyunzhu/Documents/R_workshop")
> # import the numeric data
> data <- read.table("GSE7191-data.txt", header=TRUE, row.names=1)
> # import the design info
> design <- read.table("gse7191-design.txt", header=TRUE, row.names=1)
> View(data)
> 
```

Figure 1:UI of Rstudio

Create a new project

- Demo of steps
- Console utility
- Save your work (scripts & R objects) for reproducibility

Pre-processing data: import and inspect data

Pre-processing your Data: Importing data

- The raw data usually consists of the numeric data and a description for the experimental design pertaining to the numeric data.
- Please download the data and description files from:
  - Data: TODO(insert link)
  - Description of data: TODO(insert link)
- Let's import the data by typing the command below into the console, and press enter:

```r
# set your working directory before retrieving the files
setwd("/Users/jingyunzhu/Documents/R_workshop")
```

```
## Error in setwd("/Users/jingyunzhu/Documents/R_workshop"): cannot change work
```

```r
# import the numeric data
data <- read.table("data/GSE7191-data.txt", header=TRUE, row.names=1)
# import the design info
design <- read.table("data/gse7191-design.txt", header=TRUE, row.names=1)
```

Pre-processing your Data: Inspect data

- `read.table()` renders the imported data in a `data.frame`
- To view the data.frame instances, you can click on the `data` and `design` icon inside the Environment panel on your RHS.

```
View(data)
View(design)
```

- What's their dimension size?

```
dim(data) # try ncol(), or nrow()
```

```
## [1] 12422    50
```

```
dim(design)
```

```
## [1] 50  4
```

Take a look at the structure of data

```r
str(data)
```

```
'data.frame':   12422 obs. of  50 variables:
 $ GSM172927: num  4.28 6.71 5.3 5.94 6.55 ...
 $ GSM172928: num  4.12 6.78 5.24 5.82 6.61 ...
 $ GSM172929: num  4.52 6.49 4.93 5.99 6.57 ...
 $ GSM172930: num  4.22 6.45 4.87 5.92 6.51 ...
 $ GSM172931: num  4.39 6.68 5.23 6.11 6.59 ...
 $ GSM172932: num  4.23 6.56 5.01 5.95 6.85 ...
 $ GSM172933: num  4.33 6.67 5.13 6.05 6.86 ...
 $ GSM172934: num  4.3 6.48 5.21 5.91 6.64 ...
```

Take a look at the structure of design

```r
str(design)
```

```
## 'data.frame':    50 obs. of  4 variables:
##  $ DateRun    : Factor w/ 8 levels "01/16/04","03/11/04",..: 5 5 5 5 6 4 4 6
##  $ Genotype   : Factor w/ 3 levels "S1P2_KO","S1P3_KO",..: 3 3 3 3 3 3 3 3 3
##  $ BrainRegion: Factor w/ 2 levels "hippocampus",..: 2 2 2 2 2 2 2 2 2 2 ...
##  $ Sex        : Factor w/ 2 levels "female","male": 2 2 2 2 2 1 1 1 1 1 ...
```

How are the samples assigned based on the experimental design?

- Based on each of the categorical factors in the design:

```
summary(design)
```

```
##      DateRun       Genotype       BrainRegion     Sex
##  08/14/03:8   S1P2_KO  :20   hippocampus:25   female:26
##  08/21/03:8   S1P3_KO  :10   neocortex  :25   male  :24
##  01/16/04:7   Wild_type:20
##  09/11/03:7
##  10/23/03:7
##  12/18/03:5
##  (Other) :8
```

Sample distribution based on >1 categorical factors

```r
with(design, table(Genotype, BrainRegion, Sex))
```

```
## , , Sex = female
## 
##             BrainRegion
## Genotype    hippocampus neocortex
##   S1P2_KO             5         5
##   S1P3_KO             3         3
##   Wild_type           5         5
## 
## , , Sex = male
## 
##             BrainRegion
## Genotype    hippocampus neocortex
##   S1P2_KO             5         5
##   S1P3_KO             2         2
##   Wild_type           5         5
```

Alternatively, you could try `table()`

How about the distribution of the expression level in each sample?

- summary() can integrate the results that are outputted from min(), max(), range(), fivenum(), mean(), median(), quantile()

```r
summary(data)
```

```
##    GSM172927           GSM172928           GSM172929
##  "Min.   : 2.434   " "Min.   : 2.474   " "Min.   : 2.378   "
##  "1st Qu.: 4.508   " "1st Qu.: 4.445   " "1st Qu.: 4.546   "
##  "Median : 5.995   " "Median : 5.931   " "Median : 5.961   "
##  "Mean   : 6.020   " "Mean   : 5.970   " "Mean   : 5.986   "
##  "3rd Qu.: 7.372   " "3rd Qu.: 7.309   " "3rd Qu.: 7.284   "
##  "Max.   :12.710   " "Max.   :12.710   " "Max.   :12.670   "
##    GSM172930           GSM172931
##  "Min.   : 2.345   " "Min.   : 2.372   "
##  "1st Qu.: 4.538   " "1st Qu.: 4.525   "
##  "Median : 5.976   " "Median : 6.013   "
##  "Mean   : 5.990   " "Mean   : 6.020   "
##  "3rd Qu.: 7.297   " "3rd Qu.: 7.356   "
##  "Max.   :12.740   " "Max.   :12.650   "
```

Is there any NA value?

- NA values can impede your downstream data analysis
- NA value can also result in analysis errors without triggering warnings message.

```
length(which(is.na(data)==TRUE))
```

```
## [1] 0
```

Plot your data: Stripplot, Scatter plot, Density plot, Box plot, Violin plot

Plotting your Data: introduction to ggplot2

- Let's install it

```r
#install.packages('ggplot2')
library(ggplot2)
```

- Why use ggplot2?
  - Elaborated rendering of color (gradient), and automatic legends, matching publishing standard even with default setting
  - Capable of integrating complexed/diverse dimensions of data onto one single plot
  - Each plot is stored as an object, convenient for further modification, improving code reusability
  - Linear syntax, more intuitive for the graph logics
  - For more details, read here

Plotting your Data: How to decode the ggplot2 syntex?

- gg = Grammar of Graphics
- The complexed graph is composed by **layers** that superimpose one by one, with each layer corresponding to data, coordinates, statistical representation, and etc.
    - Example: `(p <- ggplot(nDat, aes(crabHammer, geneExp, color = probeset)) + geom_point() + stat_smooth(se = F, aes(group = 1)))`
- The data (entries) can be mapped to **Aesthetics** elements, such as by their position on user-defined x- and y- axis, or to color, and linetypes.
- **Geometries** elements are used to plot the graphs

Figures plotted via ggplot2

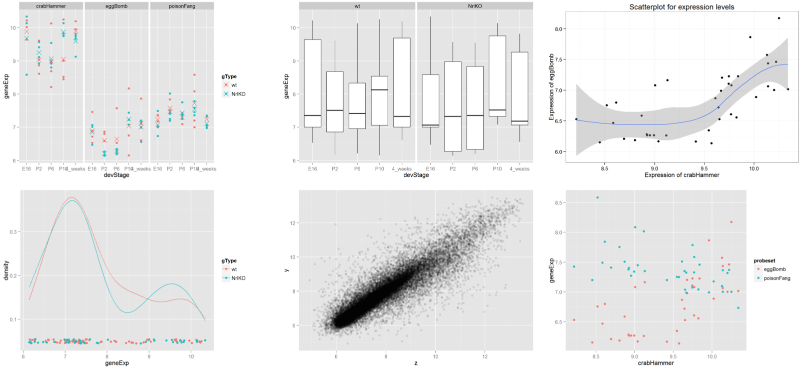


Figure 2:Examples

Plotting your Data: draw the data of some probes via stripplot (ggplot2)

Recall that:

- There are 50 samples with each containing 12422 probe expression in `data`
- There are 50 samples with each containing 4 categorical description in `design`

1. Let's shrink the `data` by picking only three probes,

- i.e. "104099_at", "99071_at", "94067_at"

```
sProbes <- c("104099_at", "99071_at", "94067_at")
sData <- data[sProbes, ]
str(sData, max.level=0)
```

```
## 'data.frame':    3 obs. of  50 variables:
```

Plotting your Data: draw the data of some probes via stripplot (ggplot2)

2. Merge the `design` and `sData` into one tall `data.frame`, so the expression data of the 3 probes could be appended after each row in `design`,

```r
t.sData <- data.frame(t(sData))

# Before merging, make sure the sample order matches
identical(rownames(t.sData), rownames(design))
```

```
## [1] TRUE
```

```r
annotatedDat <- data.frame(design, t.sData)
str(annotatedDat)
```

```
## 'data.frame':    50 obs. of  7 variables:
##  $ DateRun    : Factor w/ 8 levels "01/16/04","03/11/04",..: 5 5 5 5 6 4 4 6
##  $ Genotype   : Factor w/ 3 levels "S1P2_KO","S1P3_KO",..: 3 3 3 3 3 3 3 3 3
##  $ BrainRegion: Factor w/ 2 levels "hippocampus",..: 2 2 2 2 2 2 2 2 2 2 ...
##  $ Sex        : Factor w/ 2 levels "female","male": 2 2 2 2 2 1 1 1 1 1 ...
##  $ X104099_at : num  6.29 6.2 6.46 6.55 6.31 ...
##  $ X99071_at  : num  7.28 7.26 7.25 7.35 7.11 ...
##  $ X94067_at  : num  6.17 6.15 6.14 6.33 6.19 ...
```

Notice the “X” added in front of all the probe names.

Plotting your Data: draw the data of some probes via stripplot (ggplot2)

3. Reshape annotatedDat into a taller data.frame:

- Only 1 probe expression is appended after each row
- Introduce a new categorical descriptor, probeset, to label what probe this expression is for

```
colnames(annotatedDat)
```

```
## [1] "DateRun"     "Genotype"    "BrainRegion" "Sex"           "X104099_at"
## [6] "X99071_at"   "X94067_at"
```

```
tall.annoDat <-
  with(annotatedDat,
       data.frame(DateRun, Genotype, BrainRegion, Sex,
                  probeset = factor(rep(
                  c("X104099_at", "X99071_at", "X94067_at" ),
                  each = nrow(annotatedDat))),
                  geneExp = c(X104099_at, X99071_at, X94067_at)))
```

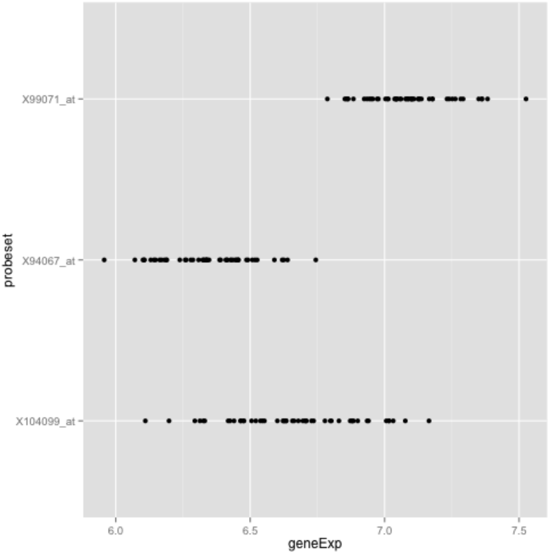
Plotting your Data: draw the data of some probes via stripplot (ggplot2)

```
str(tall.annoDat)
```

```
## 'data.frame':    150 obs. of  6 variables:
##  $ DateRun    : Factor w/ 8 levels "01/16/04","03/11/04",..: 5 5 5 5 6 4 4 6
##  $ Genotype   : Factor w/ 3 levels "S1P2_KO","S1P3_KO",..: 3 3 3 3 3 3 3 3 3
##  $ BrainRegion: Factor w/ 2 levels "hippocampus",..: 2 2 2 2 2 2 2 2 2 2 ...
##  $ Sex        : Factor w/ 2 levels "female","male": 2 2 2 2 2 1 1 1 1 1 ...
##  $ probeset   : Factor w/ 3 levels "X104099_at","X94067_at",..: 1 1 1 1 1 1
##  $ geneExp    : num  6.29 6.2 6.46 6.55 6.31 ...
```

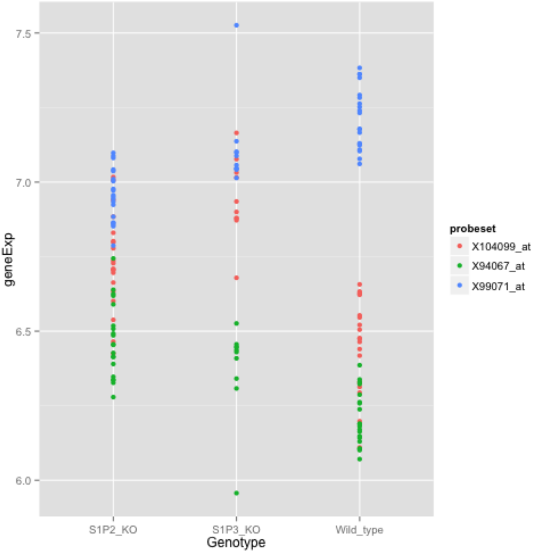
Stripplot: gene expression over 1 dimension, e.g. Probeset

```
(p <- ggplot(tall.annoDat, aes(geneExp, probeset)) +
   geom_point())
```



Stripplot: gene expression over 2 dimension, e.g. Probeset + Genotype

```
(p <- ggplot(tall.annoDat, aes(Genotype, geneExp)) +
   geom_point()+aes(color = probeset))
```



What do we mean by ggplot2 has pretty picture?

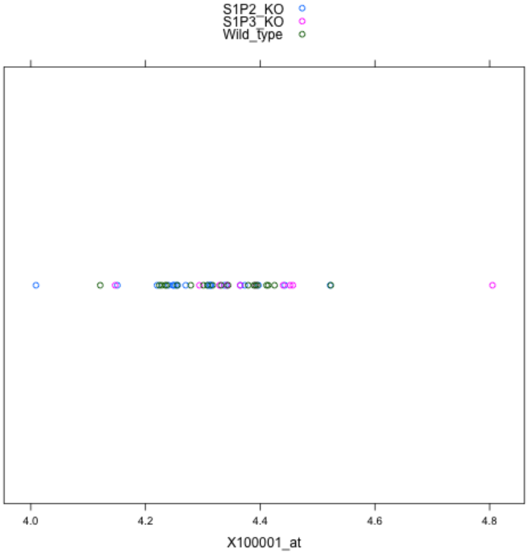
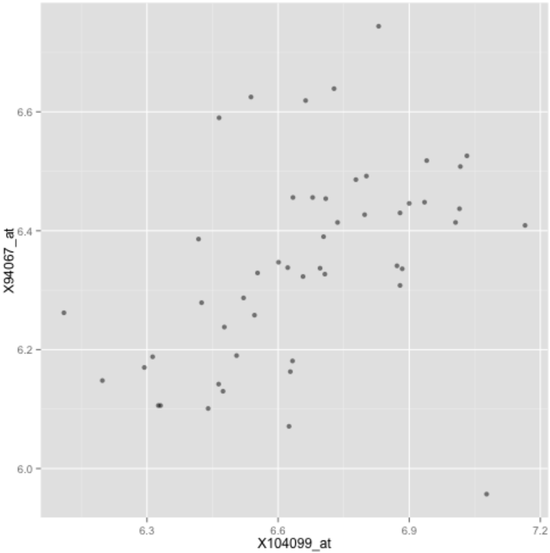


Figure 5:Lattice stripplot

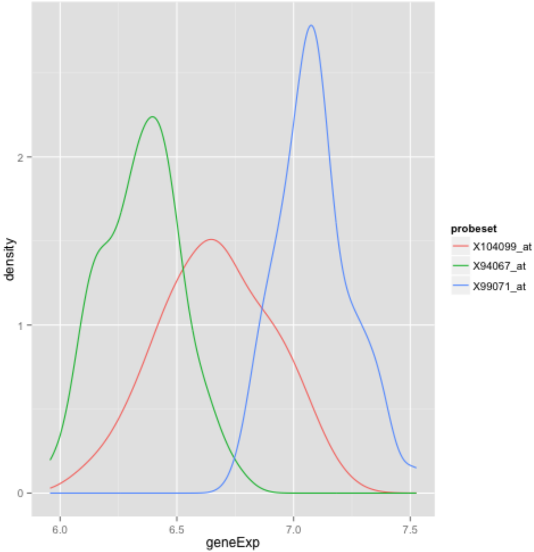
Plotting your Data: Scatterplot, X104099_at vs. X94067_at

```
p <- ggplot(annotatedDat, aes(x = X104099_at, y = X94067_at))
(p  <- p + geom_point(alpha = 0.5))
```



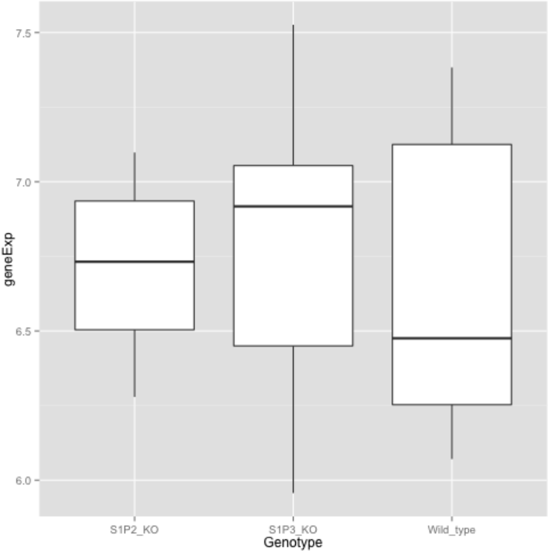
Plotting your Data: Density plot

```
(p <- ggplot(tall.annoDat, aes(geneExp, color = probeset)) +
   stat_density(geom = "line", position = "identity"))
```



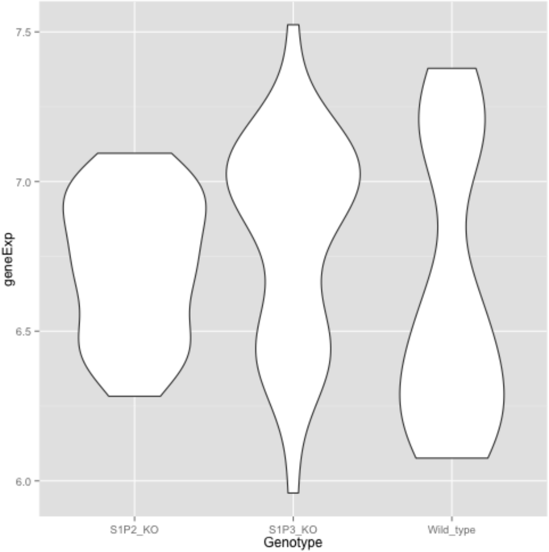
Plotting your Data: Box plot over Genotype

```
(p <- ggplot(tall.annoDat, aes(Genotype, geneExp)) +
   geom_boxplot())
```



Plotting your Data: Violin plot over Genotype

```
(p <- ggplot(tall.annoDat, aes(Genotype, geneExp)) +
   geom_violin())
```



Identify outlier sample via heatmap, pairwise correlation plot

Sample correlation

- Do we expect all the 50 samples to be clustered closely (based on the expression level of the 12422 probes) together? If no, why?
- Different experimental condition, such as treatment
- Heterogeneity of the organisms
    - genotype underlying the organisms

```
levels(design$Genotype)
```

```
## [1] "S1P2_KO"   "S1P3_KO"   "Wild_type"
```

- Outlier due to batch effect, technical errors

Heatmap of sample correlation: Calculate correlation matrix

- convert DateRun column into Date format so as to sort the 50 samples by DateRun in the increasing order

```r
annotatedDat$DateRun<-as.Date(annotatedDat$DateRun, format="%m/%d/%y")
annotatedDat <- annotatedDat[order(annotatedDat$DateRun),]
```

- convert gene expression data from data.frame into matrix

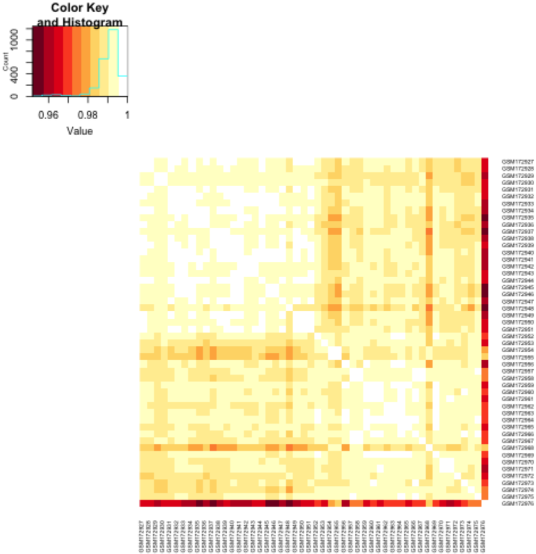
```r
mData <- as.matrix(data)
```

- construct the correlation matrix

```r
library(RColorBrewer)
cols<-c(rev(brewer.pal(9,"YlOrRd")), "#FFFFFF")
sampleCorrelation <- cor(mData)
```

Heatmap of sample correlation: Plot heatmap

```
library(gplots)
heatmap.2(sampleCorrelation, Rowv=NA, Colv=NA, symm=T,trace="none",
          dendrogram="none", col=cols, cexCol=0.5,cexRow=0.5)
```



Inspecting the correlation heatmap: seemingly an outlier?

Does sample GSM172976 deviate from the other samples? Why?

Sample correlation

- Do we expect all the 50 samples to be clustered closely (based on the expression level of the 12422 probes) together? If no, why?
- Different experimental condition, such as treatment
- Heterogeneity of the organisms
    - genotype underlying the organisms

```
levels(design$Genotype)
```

```
## [1] "S1P2_KO"   "S1P3_KO"   "Wild_type"
```

- **Outlier due to batch effect, technical errors**

Comparison of samples within the same experimental condition

- Let's inspect it more closely with a pairwise correlation plot
  - i.e. compare the outlier sample vs the samples in the same experimental condition.
- what's the experimental condition of the outlier?

```
design["GSM172976", ]
```

```
##             DateRun Genotype BrainRegion    Sex
## GSM172976 01/16/04  S1P3_KO hippocampus female
```

Comparison of samples within the same experimental condition

- find the remaining sample in this experimental group:

```
(outlierIndex <- which(colnames(data)=="GSM172976"))
```

```
## [1] 50
```

```
(allIndex <- which(design$Genotype=="S1P3_KO" &
design$BrainRegion == "hippocampus" &
design$Sex == "female"))
```

```
## [1] 48 49 50
```

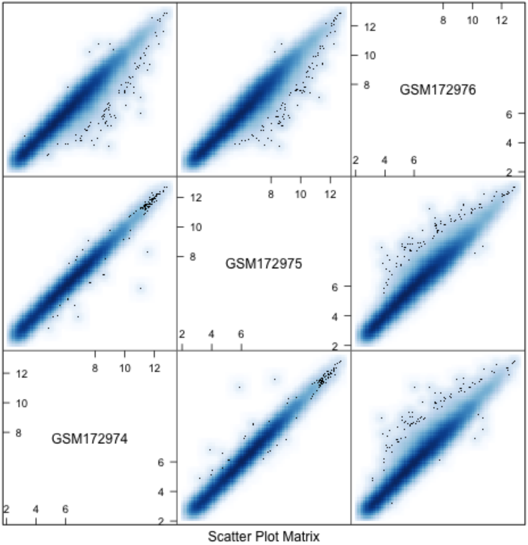
```
colnames(data)[allIndex]
```

```
## [1] "GSM172974" "GSM172975" "GSM172976"
```

```
outlierGroup <- data[,allIndex]
```

Plot the pairwise correlation of the outlier experimental group

```
library(lattice)
splom(data.frame(outlierGroup), panel = panel.smoothScatter, raster =TRUE)
```



Credits

This Workshop Brought to You By. . .

Course Developers:

- Kieran O’Neill
- Eva Yap
- Alice Zhu

Starting Material:

Much material was reused from Software Carpentry’s Bootcamp workshops and from Andy Teucher’s short R course, both under the terms of the Creative Commons Attribution License.

Reference

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