表达谱基本分析及查询

> 日期：2022-12-7
>
> 实验者：生信 2001 张子栋
>
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实验目的

1. 熟悉表达谱数据库的查询和数据下载
2. 熟悉芯片表达谱数据分析的一般流程
3. 掌握表达差异分析和基因富集分析的方法
4. 了解常用的数据可视化方法

实验内容

1. GEO 数据库查询和数据下载
2. 使用 R 包 `limma` 进行差异表达分析
3. 使用 R 包 `clusterProfiler` 进行基因富集分析
4. 使用 `gplots`，`ggpubr`，`pheatmap` 等 R 包对差异表达和富集分析进行结果可视化

实验步骤

以 `GSE46456` 为例，该实验使用的芯片平台为 GPL198，拟南芥样本基因型包括：野生型、BRI1 单突变型、GUL2 单突变型、BRI 和 GUL 双突变型，每种基因型设置**三种重复**。研究三种突变型样本与 WT 野生型样本哪些基因存在显著的差异表达。根据所提供的演示代码和相关文件，请完成以下任务：

1. 对获得的芯片数据进行数据标准化、探针过滤、limma 差异分析，写明每一步骤的代码、目的以及中间结果。

加载 R 包

```
library(cluster)
library(kohonen)
library(gplots)
library(RankProd)
library(affy)
library(affyPLM)
library(RColorBrewer)
library(limma)
library(pheatmap)
library(Mfuzz)
library(clusterProfiler)
library(enrichplot)
library(ggplot2)
library("org.At.tair.db", character.only = TRUE)
```

> 删除了导包的输出

读取数据并标准化

```
# 生成文件列表以便批量导入文件
cels <- list.files("C:\\Users\\ZidongZh\\Documents\\BioInf\\GSE46456_RAW", pattern = "*.gz", full.names = TRUE)
# 使用 Affy 包中 ReadAffy 函数，读取 CEL 文件，将其处理成 AffyBatch 对象
celfiles <- ReadAffy(filenames = cels)
# 将 AffyBatch 对象转换为 ExpressionSet 对象，对数据进行标准化
celfiles.rma <- rma(celfiles)
cols <- brewer.pal(8, "Set1")
```

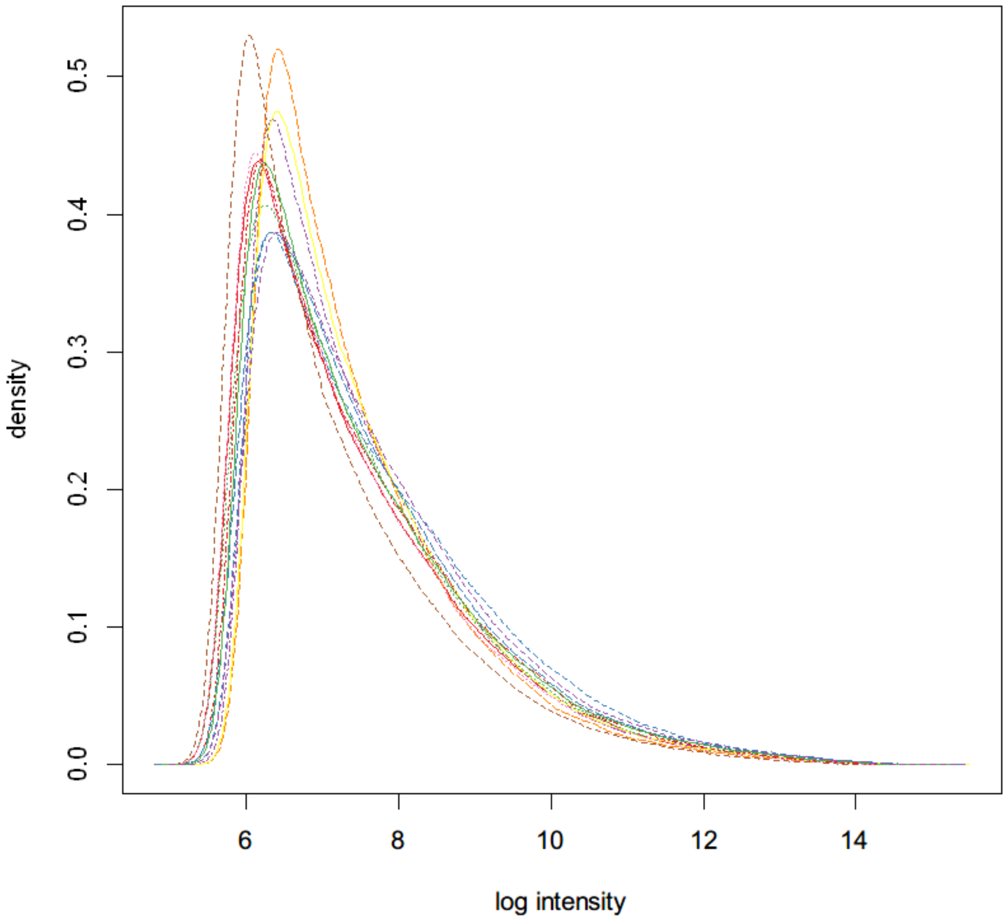
```
Warning message:
"replacing previous import 'AnnotationDbi::tail' by 'utils::tail' when loading 'ath1121501cdf'"
Warning message:
"replacing previous import 'AnnotationDbi::head' by 'utils::head' when loading 'ath1121501cdf'"
```

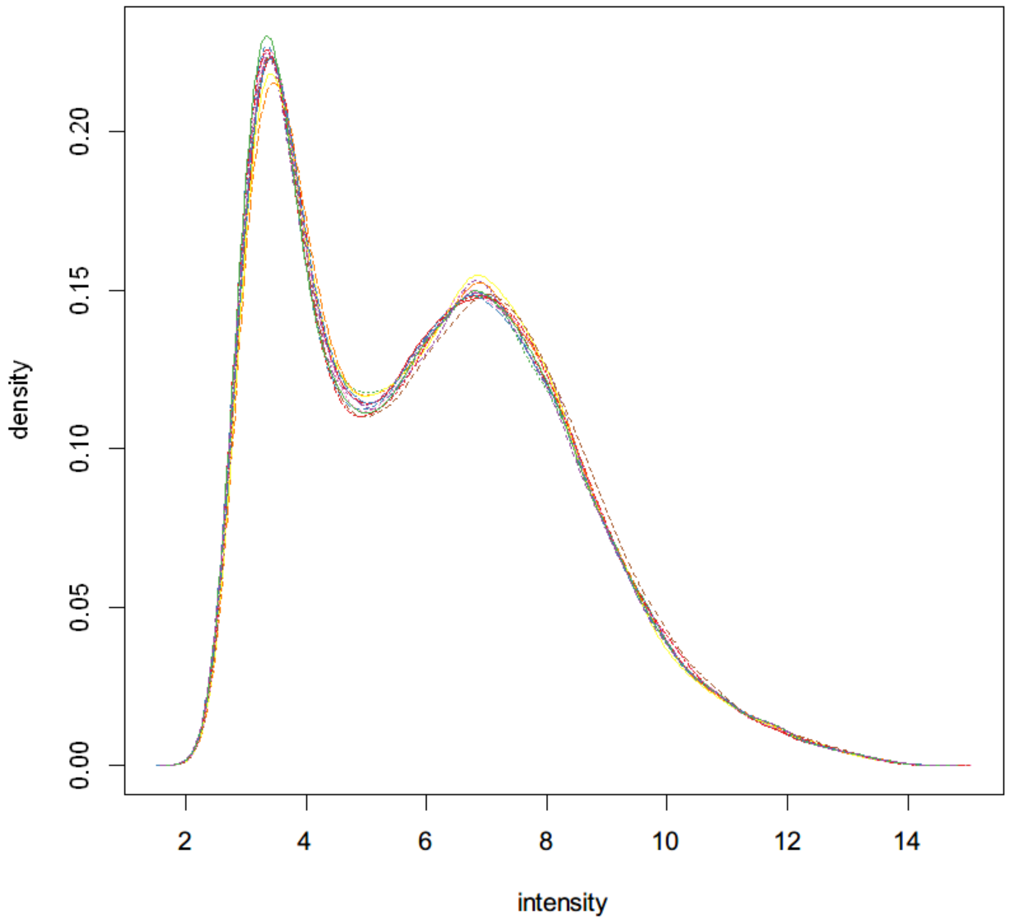
```
Background correcting
Normalizing
Calculating Expression
```

```
setwd("C:\\Users\\ZidongZh\\Documents\\BioInf\\GSE46456_RAW")
# 未标准化的数据
boxplot(celfiles, col=cols)
# 标准化的数据
boxplot(celfiles.rma, col=cols)
```

密度和对数强度直方图

```
# 未标准化的数据
hist(celfiles, col = cols)
# 标准化的数据
hist(celfiles.rma, col = cols)
```





探针过滤

```
# list 中的 eset 为过滤后的 ExpressionSet，filter.log 为每一步过滤到多少探针的记录。
library(genefilter)
celfiles.filtered <- nsFilter(celfiles.rma, require.entrez=FALSE, remove.dupEntrez=FALSE)
```

```
celfiles.filtered$filter.log
celfiles.filtered$eset
```

```
$numLowVar
      11373
$feature.exclude
      64
```

```
ExpressionSet (storageMode: lockedEnvironment)
assayData: 11373 features, 12 samples
  element names: exprs
protocolData
  sampleNames: GSM1130596_Ws-2-1.CEL.gz GSM1130597_Ws-2-2.CEL.gz ...
    GSM1130607_gul2-1bri1-5-3.CEL.gz (12 total)
  varLabels: ScanDate
  varMetadata: labelDescription
phenoData
  sampleNames: GSM1130596_Ws-2-1.CEL.gz GSM1130597_Ws-2-2.CEL.gz ...
    GSM1130607_gul2-1bri1-5-3.CEL.gz (12 total)
  varLabels: sample
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: ath1121501
```

获得表达量矩阵

```r
eset <- exprs(celfiles.filtered$eset)
head(eset)
```

A matrix: 6 × 12 of t

	GSM1130596_Ws-2-1.CEL.gz	GSM1130597_Ws-2-2.CEL.gz	GSM1130598_Ws-2-3.CEL.gz	GSM1130599_bri1-5-1.CEL.gz	GSM1130600_bri1-5-2.CEL.gz	GSM1130601_bri1-5-3.CEL.gz
244901_at	5.224648	5.428151	5.546510	4.683135	4.753393	4.463033
244902_at	5.149407	5.187442	4.886097	4.672779	4.805556	4.794880
244903_at	5.592680	5.436074	5.638751	5.562216	5.622267	5.224591
244904_at	4.985820	5.072172	5.262937	5.016912	5.446725	5.482161
244906_at	5.727308	5.889640	5.323069	5.381804	5.609199	5.514687
244912_at	6.465566	6.586064	6.814510	7.653073	7.871753	8.260488

增加探针对应的基因信息

```r
araAnno <- read.delim("C:\\Users\\ZidongZh\\Documents\\BioInf\\affy_ATH1_array_elements-2010-12-20.txt")
head(araAnno)
head(eset)
ids <- match(rownames(eset), araAnno$array_element_name)
length(araAnno$array_element_name)
length(ids)
# ids
head(eset)
rownames(eset) <- araAnno$locus[ids]
colnames(eset) <- sub(".CEL.gz", "", colnames(eset))
head(eset)
```

A data.frame: 6 × 9

	array_element_name	array_element_type	organism	is_control	locus	description	chromosome	start
	<chr>	<chr>	<chr>	<chr>	<chr>	<chr>	<chr>	<chr>
1	244901_at	oligonucleotide	Arabidopsis thaliana	no	ATMG00640	hydrogen ion transporting ATP synthases, rotational mechanism;zinc ion binding	M	188160
2	244902_at	oligonucleotide	Arabidopsis thaliana	no	ATMG00650	NADH dehydrogenase subunit 4L	M	188954
3	244903_at	oligonucleotide	Arabidopsis thaliana	no	ATMG00660	hypothetical protein	M	190106
4	244904_at	oligonucleotide	Arabidopsis thaliana	no	ATMG00670	hypothetical protein	M	191055
5	244905_at	oligonucleotide	Arabidopsis thaliana	no	ATMG00680	hypothetical protein	M	201768
6	244906_at	oligonucleotide	Arabidopsis thaliana	no	ATMG00690	hypothetical protein	M	203634

A matrix: 6 × 12 of t

	GSM1130596_Ws-2-1.CEL.gz	GSM1130597_Ws-2-2.CEL.gz	GSM1130598_Ws-2-3.CEL.gz	GSM1130599_bri1-5-1.CEL.gz	GSM1130600_bri1-5-2.CEL.gz	GSM1130601_bri1-5-3.CEL.gz
244901_at	5.224648	5.428151	5.546510	4.683135	4.753393	4.463033
244902_at	5.149407	5.187442	4.886097	4.672779	4.805556	4.794880
244903_at	5.592680	5.436074	5.638751	5.562216	5.622267	5.224591
244904_at	4.985820	5.072172	5.262937	5.016912	5.446725	5.482161
244906_at	5.727308	5.889640	5.323069	5.381804	5.609199	5.514687
244912_at	6.465566	6.586064	6.814510	7.653073	7.871753	8.260488

22810

11373

A matrix: 6 × 12 of t

	GSM1130596_Ws-2-1.CEL.gz	GSM1130597_Ws-2-2.CEL.gz	GSM1130598_Ws-2-3.CEL.gz	GSM1130599_bri1-5-1.CEL.gz	GSM1130600_bri1-5-2.CEL.gz	GSM1130601_bri1-5-3.CEL.gz
244901_at	5.224648	5.428151	5.546510	4.683135	4.753393	4.463033
244902_at	5.149407	5.187442	4.886097	4.672779	4.805556	4.794880
244903_at	5.592680	5.436074	5.638751	5.562216	5.622267	5.224591
244904_at	4.985820	5.072172	5.262937	5.016912	5.446725	5.482161
244906_at	5.727308	5.889640	5.323069	5.381804	5.609199	5.514687
244912_at	6.465566	6.586064	6.814510	7.653073	7.871753	8.260488

A matrix: 6 ×

	GSM1130596_Ws-2-1	GSM1130597_Ws-2-2	GSM1130598_Ws-2-3	GSM1130599_bri1-5-1	GSM1130600_bri1-5-2	GSM113 5-3
ATMG00640	5.224648	5.428151	5.546510	4.683135	4.753393	4.46303
ATMG00650	5.149407	5.187442	4.886097	4.672779	4.805556	4.79488
ATMG00660	5.592680	5.436074	5.638751	5.562216	5.622267	5.22459
ATMG00670	4.985820	5.072172	5.262937	5.016912	5.446725	5.48216
ATMG00690	5.727308	5.889640	5.323069	5.381804	5.609199	5.51468
AT2G07783;ATMG00830	6.465566	6.586064	6.814510	7.653073	7.871753	8.26048

3. 运用 `limma` 获得突变体和野生型的差异表达基因集，并阐述差异分析结果的各列含义。

分组矩阵

```
group_list <- c(rep('wild_type', 3),
                rep('bri1.5_mutant', 3),
                rep('gul2.1_mutant', 3),
                rep('gul2.1_bri1.5_mutant',3))
design <- model.matrix(~0+factor(group_list))
colnames(design) <- levels(factor(group_list))
rownames(design) <- colnames(eset)
design
```

A matrix: 12 × 4 of type dbl

	bri1.5_mutant	gul2.1_bri1.5_mutant	gul2.1_mutant	Wild_type
GSM1130596_Ws-2-1	0	0	0	1
GSM1130597_Ws-2-2	0	0	0	1
GSM1130598_Ws-2-3	0	0	0	1
GSM1130599_bri1-5-1	1	0	0	0
GSM1130600_bri1-5-2	1	0	0	0
GSM1130601_bri1-5-3	1	0	0	0
GSM1130602_gul2-1-1	0	0	1	0
GSM1130603_gul2-1-2	0	0	1	0
GSM1130604_gul2-1-3	0	0	1	0
GSM1130605_gul2-1bri1-5-1	0	1	0	0
GSM1130606_gul2-1bri1-5-2	0	1	0	0

	bri1.5_mutant	gul2.1_bri1.5_mutant	gul2.1_mutant	Wild_type
GSM1130607_gul2-1bri1-5-3	0	1	0	0

构建对照矩阵

```
contrast.matrix <- makeContrasts(bri1.5_mutant-Wild_type,
                                 gul2.1_mutant-Wild_type,
                                 gul2.1_bri1.5_mutant-Wild_type,
                                 levels = design)
contrast.matrix
```

A matrix: 4 × 3 of type dbl

	bri1.5_mutant - Wild_type	gul2.1_mutant - Wild_type	gul2.1_bri1.5_mutant - Wild_type
bri1.5_mutant	1	0	0
gul2.1_bri1.5_mutant	0	0	1
gul2.1_mutant	0	1	0
Wild_type	-1	-1	-1

拟合 差值计算 检验

```
# limma
# 线性模型拟合
fit1 <- lmFit(eset, design)
# 根据对比模型进行差值计算
fit2 <- contrasts.fit(fit1, contrast.matrix)
# 贝叶斯检验
fit2 <- eBayes(fit2)
```

输出差异表达基因

```
# 利用 toptable 导出 DEG 结果
limma_results <- lapply(colnames(contrast.matrix),
                        function(x) {
                          topTable(fit2,
                                   coef     = x,
                                   adjust   = "fdr",
                                   sort.by  = "logFC",
                                   number   = Inf)
                        })
length(limma_results)
# 对导出的结果标记 title 信息
names(limma_results) <- colnames(contrast.matrix)
head(limma_results[[1]])
save(limma_results, file = "limma_compare_res.RData")
# 对每对比较的样本对 DEG 结果单独导出 DEG 信息 6
for (n in names(limma_results)) {
    write.table(limma_results[[n]],
                file        = sprintf("%s.tsv", gsub(' ', '', n)),
                row.names   = FALSE,
                sep         = "\t")
}
save(eset, file = "eset.RData")
head(eset)
```

3

A data.frame: 6 × 7

	ID	logFC	AveExpr	t	P.Value	adj.P.Val	B
	<chr>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
311	AT4G15620	4.414308	5.380715	35.58470	2.706107e-19	1.538828e-15	33.49616
46	ATCG00790	4.022998	10.302096	35.58878	2.700114e-19	1.538828e-15	33.49803
1693	AT5G53870	-3.405975	6.478671	-20.55259	9.726000e-15	7.374253e-12	23.92538
742	AT1G57750	-3.346731	5.462349	-29.17670	1.241947e-17	3.531167e-14	30.16103
45	ATCG00780	3.195997	8.690368	21.39171	4.579686e-15	4.539751e-12	24.65109
21	ATCG00065	3.039406	7.137484	22.37312	1.963188e-15	2.480816e-12	25.46182

A matrix: 6 ×

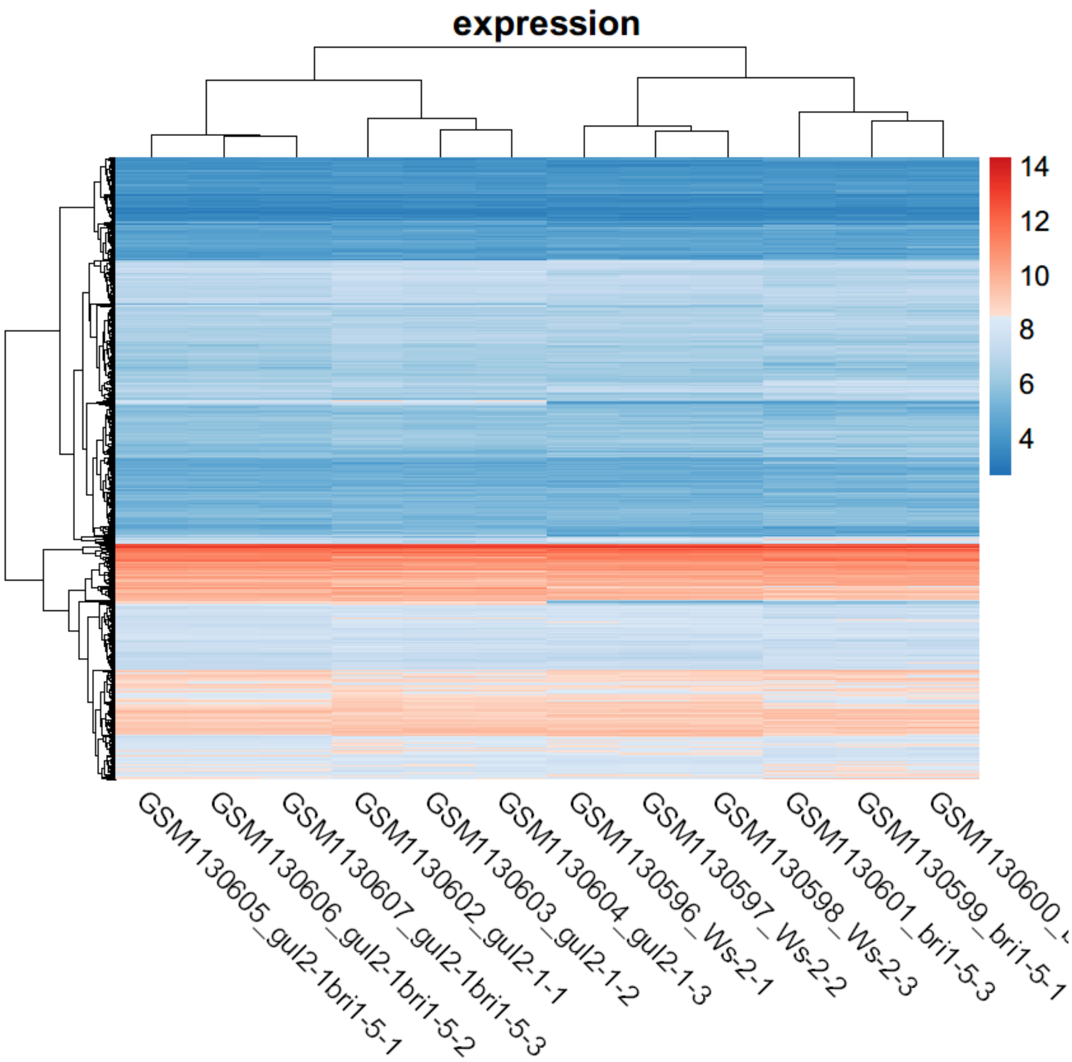
	GSM1130596_Ws-2-1	GSM1130597_Ws-2-2	GSM1130598_Ws-2-3	GSM1130599_bri1-5-1	GSM1130600_bri1-5-2	GSM113 5-3
ATMG00640	5.224648	5.428151	5.546510	4.683135	4.753393	4.46303
ATMG00650	5.149407	5.187442	4.886097	4.672779	4.805556	4.79488
ATMG00660	5.592680	5.436074	5.638751	5.562216	5.622267	5.22459
ATMG00670	4.985820	5.072172	5.262937	5.016912	5.446725	5.48216
ATMG00690	5.727308	5.889640	5.323069	5.381804	5.609199	5.51468
AT2G07783;ATMG00830	6.465566	6.586064	6.814510	7.653073	7.871753	8.26048

- `ID`: Gene ID
- `logFC`: 两组表达值之间以2为底对数化的变化倍数（Fold change, FC），由于基因表达矩阵本身已经取了对数，这里实际上只是两组基因表达值均值之差。
- `AveExpr`: 该探针组所在所有样品中的平均表达值。
- `t`: 贝叶斯调整后的两组表达值间 T 检验中的 t 统计量。
- `P.Value`: 检验 P 值。
- `adj.P.Val`: 调整后的 P 值。（多重检验 BH 等方法）
- `B`: 是经验贝叶斯得到的标准差的对数化值。

差异表达分析结果可视化

DEG plot

```
pheatmap(eset,
         col                = c(colorRampPalette(brewer.pal(9, "Blues")[7:2])(100),
                                colorRampPalette(brewer.pal(9, "Reds")[2:7])(100)),
         border_color       = NA,
         cluster_rows       = T,
         cluster_cols       = T,
         show_rownames      = F,
         show_colnames      = T,
         angle_col          = 315,
         fontsize           = 13,
         main               = "expression",
         display_numbers    = F)
```



DEG 火山图

```
library(ggpubr)
library(ggthemes)
deg.data <- read.table("C:\\Users\\ZidongZh\\Documents\\BioInf\\GSE46456_RAW\\bri1.5_mutant-Wild_type.tsv", header = T, sep = "\t")
# - log10 值转换
deg.data$logP <- -log10(deg.data$adj.P.Val)
# 定义 Group 列
deg.data$Group <- "not-significant"
# 定义 DEG 标准
deg.data$Group[which ((deg.data$adj.P.Val < 0.05) & (deg.data$logFC > 2))] <- "up-regulated"
# 定义 DEG 标准
deg.data$Group[which ((deg.data$adj.P.Val < 0.05) & (deg.data$logFC < -2))] <- "down-regulated"
# 统计 DEG 数量
table(deg.data$Group)
ggscatter(deg.data, x = "logFC", y = "logP", color = "Group") + theme_base()
```

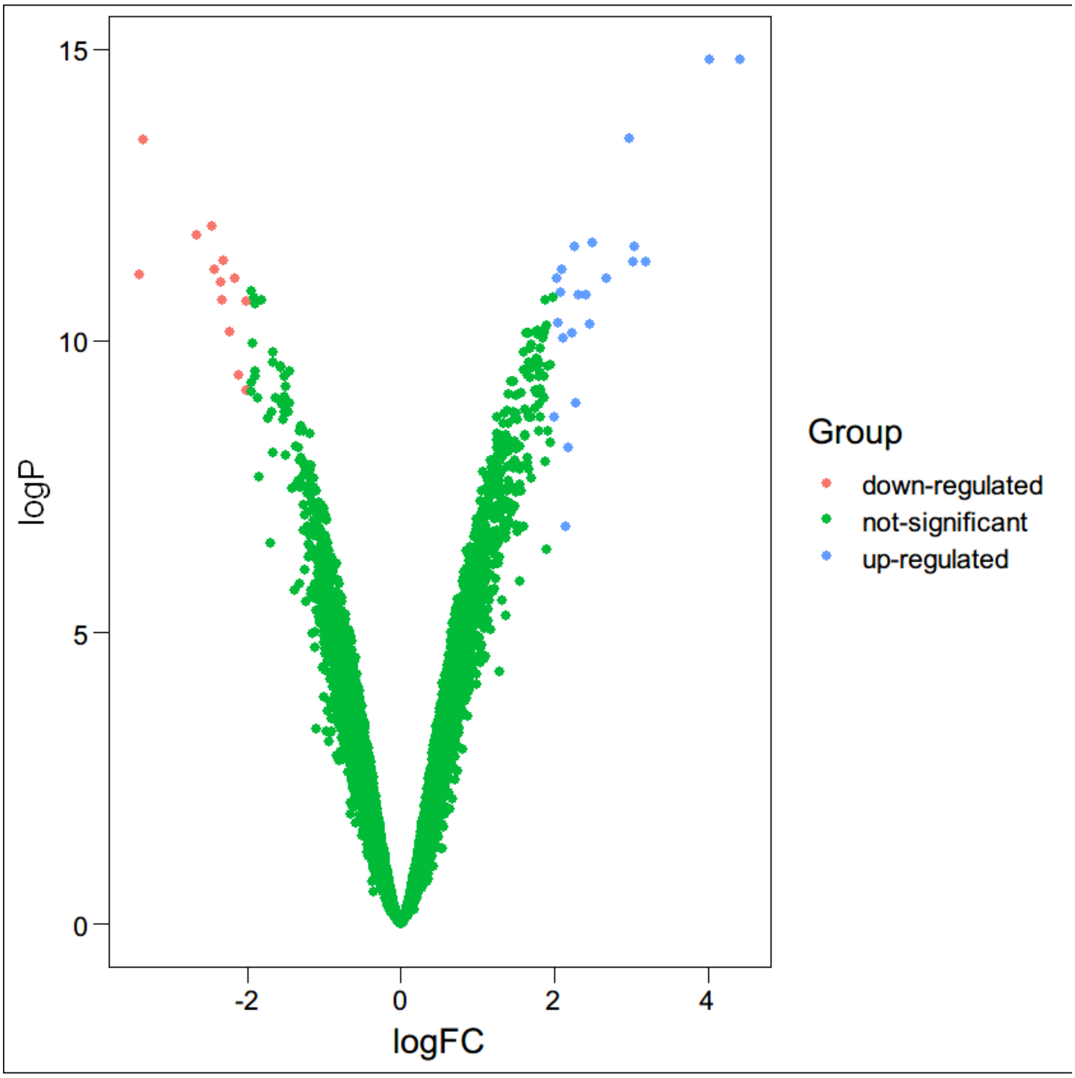
```
Warning message:
"程辑包'ggpubr'是用R版本4.1.3 来建造的"

载入程辑包：'ggpubr'
```

The following object is masked from 'package:enrichplot':

```
    color_palette
```

Warning message:
"程辑包'ggthemes'是用R版本4.1.3 来建造的"

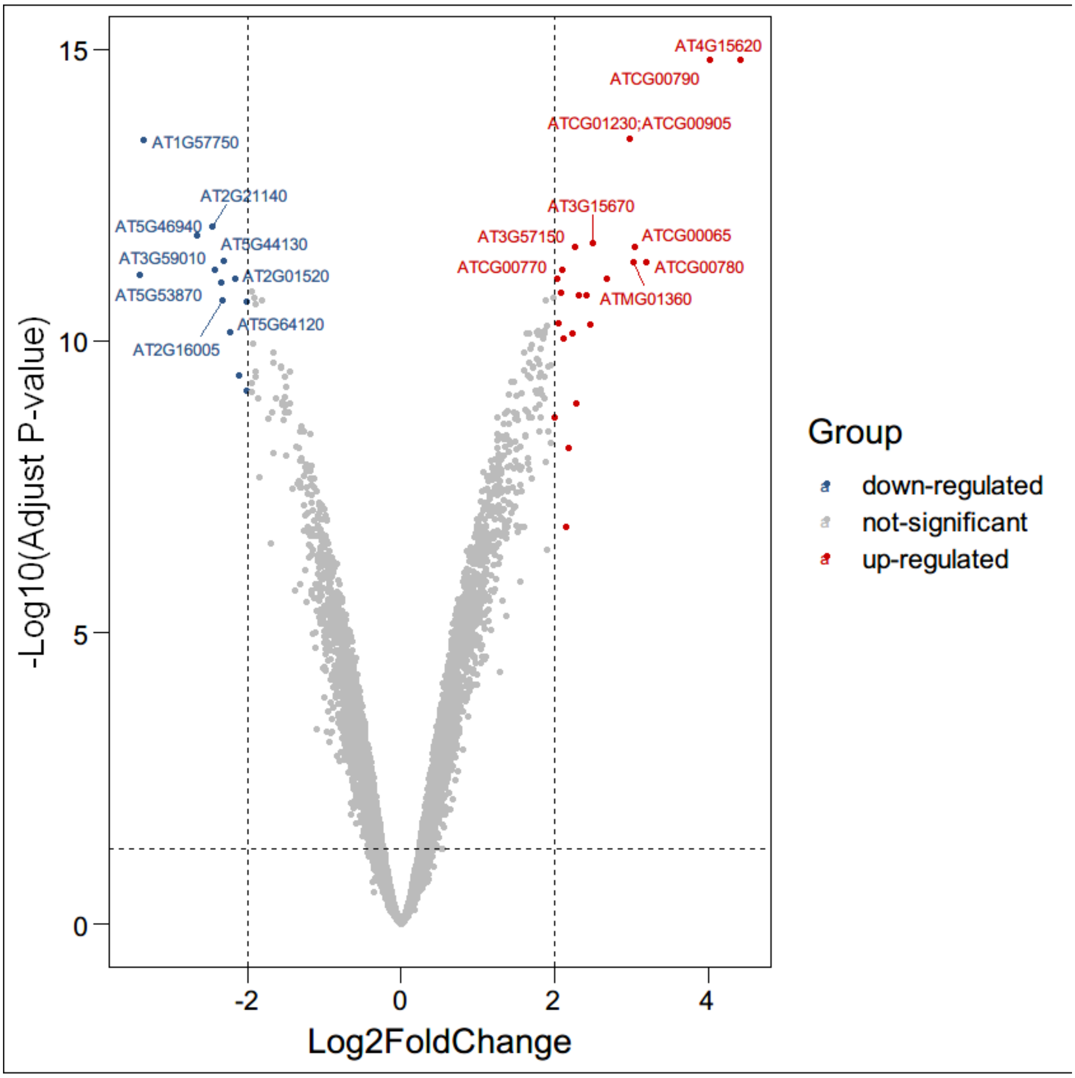
```
down-regulated not-significant    up-regulated
            13           11338            22
```



```
# 新加一列 lable
deg.data$Lable <- ""
# 对差异表达基因 P 值从小到大排序
deg.data <- deg.data[order(deg.data$adj.P.Val), ]
# 从高表达基因中选取 adj.P.Val 最显著的 10 个基因
up.genes <- head(deg.data$ID[which(deg.data$Group == "up-regulated")], 10)
# 从低表达基因中选取 adj.P.Val 最显著的 10 个基因
down.genes <- head(deg.data$ID[which(deg.data$Group == "down-regulated")], 10)
# 讲上两步选取的显著基因合并并加入到 lable 中
deg.top10.genes <- c(as.character(up.genes), as.character(down.genes))
deg.data$Lable[match(deg.top10.genes, deg.data$ID)] <- deg.top10.genes
```

```
ggscatter(deg.data,
          x             = "logFC",
          y             = "logP",
          color         = "Group",
          palette       = c("#2f5688", "#BBBBBB", "#CC0000"),
          size          = 1,
          label         = deg.data$Lable,
          font.label    = 8,
          repel         = T,
          xlab          = "Log2FoldChange",
          ylab          = "-Log10(Adjust P-value)",) +
  theme_base() +
  geom_hline(yintercept = 1.30, linetype="dashed") +
  geom_vline(xintercept = c(-2,2),linetype="dashed")
```

```
Warning message:
"ggrepel: 8 unlabeled data points (too many overlaps). Consider increasing max.overlaps"
```



输出 DEG 结果

```
write.table(deg.data, ".\\DEG_Plot_bri1.5mutant-Wild_type.tsv", sep = "\t")
```

3. 对所有基因做 GSEA 富集分析；并对三组上调的差异表达基因(`bri1-WT`, `gul2-WT`, `bri1_gul2-WT`)做 GO 富集分析，并解释富集结果，如有图片请注明图注信息。

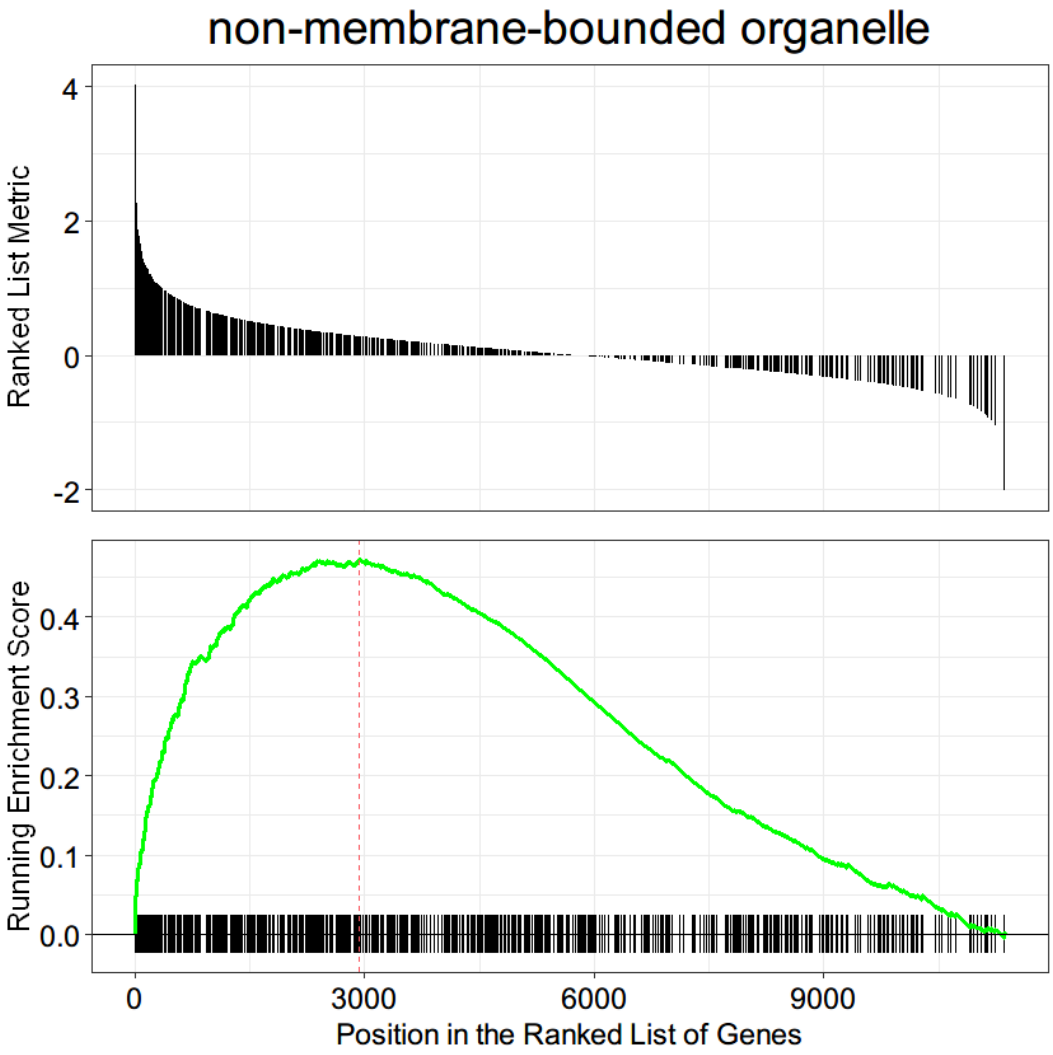
富集分析 GSEA

```
# 导入 DEG 信息
data <- read.table(".\\DEG_Plot_bri1.5mutant-Wild_type.tsv", sep = "\t", header = TRUE)
GSEA_data <- data
# 提取表达量变化值
GSEA_gene_lists <- GSEA_data$logFC
# 给提取出来的值赋予 ID
names(GSEA_gene_lists) <- GSEA_data$ID
# 降序排列
GSEA_gene_lists <- sort(GSEA_gene_lists, decreasing = TRUE)
head(GSEA_gene_lists)
```

```
AT4G15620:          4.41430828002192 ATCG00790:          4.02299842465399 ATCG00780:          3.19599709685614 ATCG00065:
        3.03940635696951 ATMG01360:          3.02028076719578 ATCG01230;ATCG00905:          2.96904529537879
```

```r
# 获取拟南芥数据库信息
organisms <- get("org.At.tair.db")
gse <- gseGO(geneList       = GSEA_gene_lists,
             ont            = "ALL",
             keyType        = "TAIR",
             nPerm          = 10000,
             minGSSize      = 3,
             maxGSSize      = 800,
             pvalueCutoff   = 0.05,
             verbose        = TRUE,
             OrgDb          = organisms,
             pAdjustMethod  = "none")
gseaplot(gse, by = "all", title = gse$Description[1], geneSetID = 1)
```

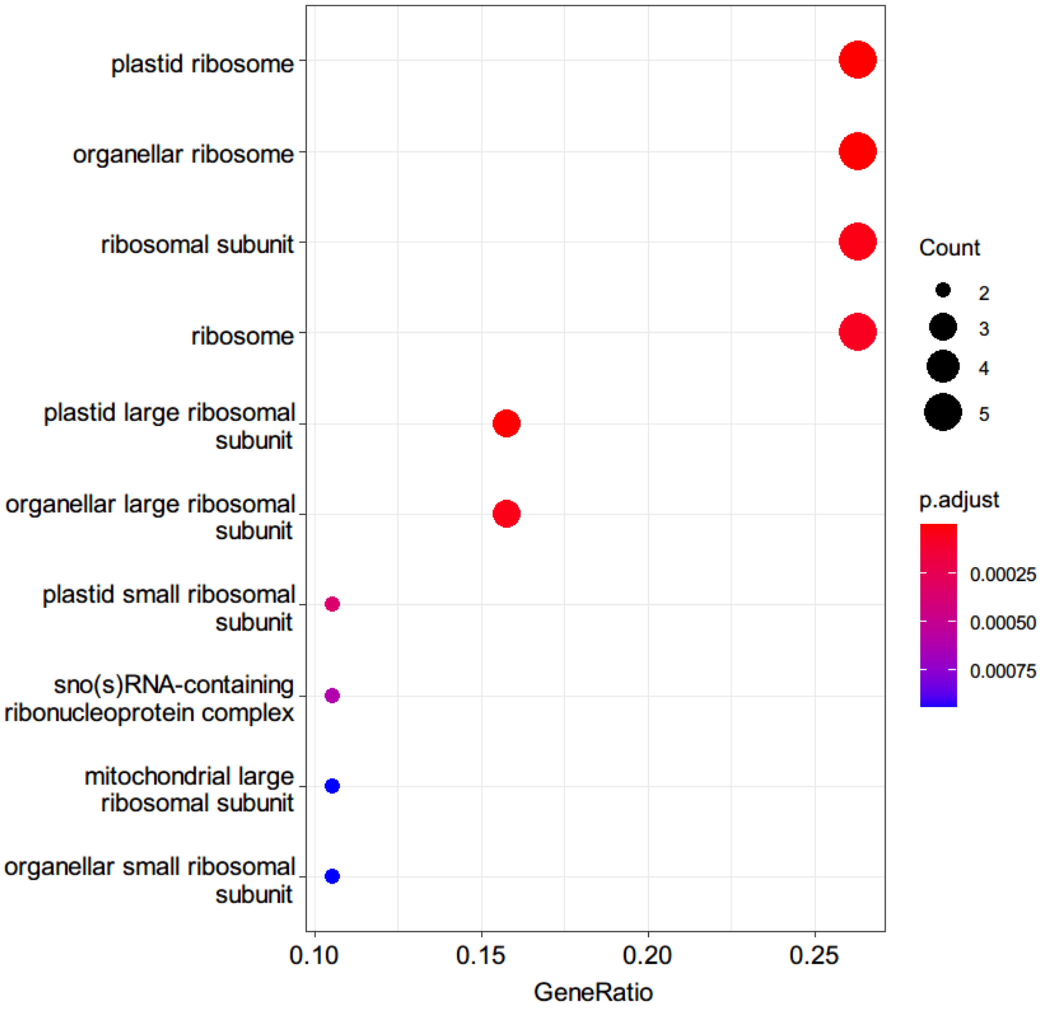
```
preparing geneSet collections...

GSEA analysis...

Warning message in .GSEA(geneList = geneList, exponent = exponent, minGSSize = minGSSize, :
"We do not recommend using nPerm parameter incurrent and future releases"
Warning message in fgsea(pathways = geneSets, stats = geneList, nperm = nPerm, minSize = minGSSize, :
"You are trying to run fgseaSimple. It is recommended to use fgseaMultilevel. To run fgseaMultilevel, you need to remove the nperm argument in the fgsea function call."
Warning message in preparePathwaysAndStats(pathways, stats, minSize, maxSize, gseaParam, :
"There are duplicate gene names, fgsea may produce unexpected results."
leading edge analysis...

done...
```



从总体上看，该基因集是上调趋势。

GO 富集分析

```r
data<- data[data$Group == "up-regulated", ]
ego <- enrichGO(gene            = data$ID,
                keyType         = "TAIR",
                OrgDb           = organisms,
                ont             = "ALL",
                pAdjustMethod   = "BH",
                qvalueCutoff    = 0.05)
dotplot(ego, showCategory = 10)
```



讨论

在这次上机实验中，熟悉并掌握了分析芯片表达数据的流程，表达差异分析和基因辅基分析的方法，了解了常用的数据可视化方式。