# Bispecific_Antibody_Target_Selection_ProstateCancer

2024-02-21

```
suppressWarnings({
case=subset(phenoDF,cancer=='prostate adenocarcinoma'&sample.type == 'primary') #Select Prostate cancer
case_id=case$sample.id #getting case IDS
control=subset(phenoDF,sample.type=='normal'&biopsy.site=='PROSTATE')#Select Normal Prostate samples
control_id=control$sample.id #getting control IDS

case_expr=loadOctadCounts(case_id,type='tpm',file='~/Downloads/octad.counts.and.tpm.h5')
case_expr=as.data.frame(case_expr)
control_expr=loadOctadCounts(control_id ,type='tpm',file='~/Downloads/octad.counts.and.tpm.h5')
control_expr=as.data.frame(control_expr)

#final data
case_with_control_expr=cbind(case_expr,control_expr)

#convert ensg to hgnc and select surface-expressed genes according to  compartments.jensenlab.org
case_with_control_expr=ensg_to_hgnc(case_with_control_expr,select_surface=TRUE)

phenotype_vector=as.factor(c(rep('case',ncol(case_expr)),rep('control',ncol(control_expr))))
})
```

```
## [1] "loading 60498 TPM expression values for 494 samples"
## [1] "loading 60498 TPM expression values for 100 samples"
```

#Perform Differential Gene Expression to filter out non-significant genes to speed up the computation

```
annotation=data.frame(sample=c(colnames(case_expr),colnames(control_expr)),phenotype=c(rep('cancer',leng
annotation$phenotype=as.factor(annotation$phenotype)
expression=DGEList(counts=as.matrix(case_with_control_expr),group=annotation$phenotype)
dim(expression)
```

```
## [1] 3736  594
```

```
keep <- rowSums(cpm(expression)>100) >= 2
expression <- expression[keep,]
dim(expression)
```

```
## [1] 3117  594
```

```
expression$samples$lib.size <- colSums(expression$counts)

suppressWarnings({
  expression <- calcNormFactors(expression)
```

```
})

expression_disp <- estimateCommonDisp(expression, verbose=T)
```

```
## Disp = 1e-04 , BCV = 0.01
```

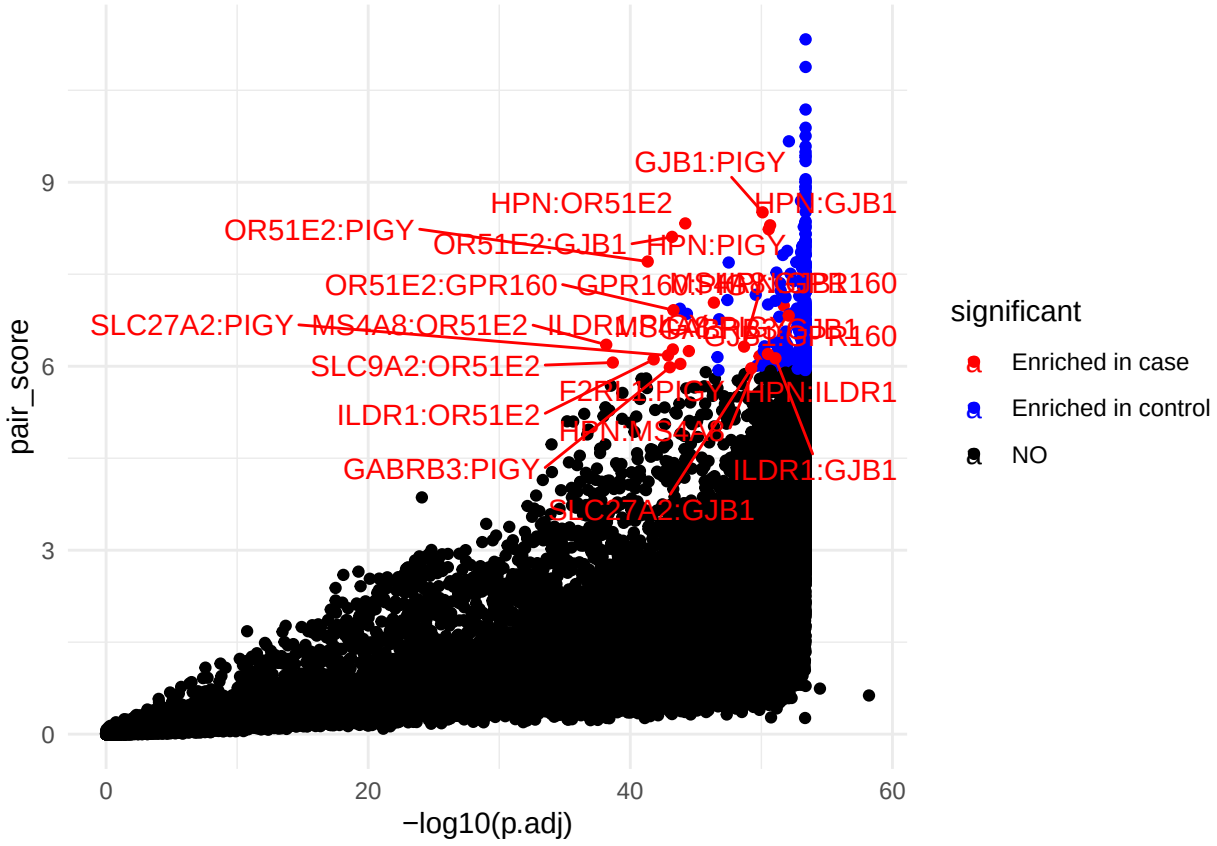
```
expression_disp <- estimateTagwiseDisp(expression_disp)
DE <- exactTest(expression_disp, pair=c(1,2)) # compare groups 1 and 2
DE=DE$table
DE$padj=p.adjust(DE$PValue,method='BH')

DE1=subset(DE,padj<0.05&abs(logFC)>1) # The cutoff criteria can be changed

#filter out only surface-expressed DE genes. Just to speed up.
case_with_control_expr=case_with_control_expr[row.names(case_with_control_expr)%in%row.names(DE1),]
dataframe_for_computation=as.data.frame(t(case_with_control_expr))

#this step takes a while
small_res=compute_bsabs(antigene_1=colnames(dataframe_for_computation),data_input=dataframe_for_computa
```

```
##   |                                                                      |
```

```
head(small_res)
```

```
##   antigen_1 antigen_2 distance   spread angle_cos pair_score      p.value
## 1    ITGA2B     SCN4A 1.266441 1.645612 0.7126953   1.485307 8.513137e-52
## 2    ITGA2B      GIPR 2.655597 1.627037 0.9112219   3.937166 8.630330e-50
## 3    ITGA2B    NPC1L1 1.327407 1.654277 0.7319016   1.607182 1.249741e-51
## 4    ITGA2B   SLC11A1 2.383090 1.665728 0.9500795   3.771416 9.820476e-55
## 5    ITGA2B   CYP3A43 1.436237 1.646023 0.8889049   2.101442 2.291172e-51
## 6    ITGA2B    SLC7A9 1.639132 1.656881 0.9921792   2.694607 1.994614e-52
##          p.adj case_greater
## 1 3.597593e-51  FALSE_FALSE
## 2 2.706100e-49  FALSE_FALSE
## 3 5.124317e-51  FALSE_FALSE
## 4 1.223267e-53  FALSE_FALSE
## 5 8.991584e-51  FALSE_FALSE
## 6 9.610274e-52  FALSE_FALSE
```

```
## PLOT FIG.2D

suppressWarnings({
plot_bsabs(small_res,label='case',pval_cut_off=0.01,pair_score_cut_off=quantile(small_res$pair_score,.9
})
```




```
#subset result table to keep only those pairs where both markers have higher expression in case than co
small_res=small_res[small_res$case_greater=="TRUE_TRUE",]

#ordering as per pair score , highest score should be at top
small_res=small_res[order(small_res$pair_score,decreasing = T),]

#Subsetting top 20 pairs
small_res_selective=small_res[c(1:20),]

# unique marker genes in top 20 pairs
marker_list=unique(c(small_res_selective$antigen_1,small_res_selective$antigen_2))
marker_list
```

```
##  [1] "GJB1"    "HPN"     "OR51E2"  "GPR160"  "MS4A8"   "ILDR1"   "GABRB3"
##  [8] "SLC27A2" "SLC9A2"  "PIGY"
```

```
# Checking the expression of marker genes in healthy tissue

healthy_tissues=subset(phenoDF,sample.type=='normal')
healthy_tissues=subset(healthy_tissues,grepl('BRAIN',biopsy.site)|biopsy.site=='LIVER'|biopsy.site=='LU

healthy_tissues <- healthy_tissues %>%mutate(biopsy.site = ifelse(grepl("BRAIN", biopsy.site), "BRAIN",
healthy_tissues <- healthy_tissues %>%mutate(biopsy.site = ifelse(grepl("HEART", biopsy.site), "HEART",
healthy_tissues <- healthy_tissues %>%mutate(biopsy.site = ifelse(grepl("KIDNEY", biopsy.site), "KIDNEY
```

```
healthy_tissues_expr=loadOctadCounts(healthy_tissues$sample.id,type='tpm',file='~/Downloads/octad.counts
```

```
## [1] "loading 60498 TPM expression values for 1950 samples"
```

```
healthy_tissues_expr=as.data.frame(healthy_tissues_expr)

healthy_tissues_expr=ensg_to_hgnc(healthy_tissues_expr,select_surface=FALSE)
healthy_tissues_expr=healthy_tissues_expr[row.names(healthy_tissues_expr)%in%marker_list,]

healthy_tissues_expr=healthy_tissues_expr[order(rownames(healthy_tissues_expr)),]

healthy_tissues_expr=as.data.frame(t(healthy_tissues_expr))

healthy_tissues_expr$Sample=healthy_tissues$biopsy.site[match(rownames(healthy_tissues_expr),healthy_tis

case_with_control_expr2=case_with_control_expr[row.names(case_with_control_expr)%in%marker_list,]

case_with_control_expr2=case_with_control_expr2[order(rownames(case_with_control_expr2)),]

case_with_control_expr2=as.data.frame(t(case_with_control_expr2))

case_with_control_expr2$Sample=ifelse(rownames(case_with_control_expr2)%in%case_id,"PROSTATE_CANCER","PR

colnames(case_with_control_expr2)==colnames(healthy_tissues_expr)
```

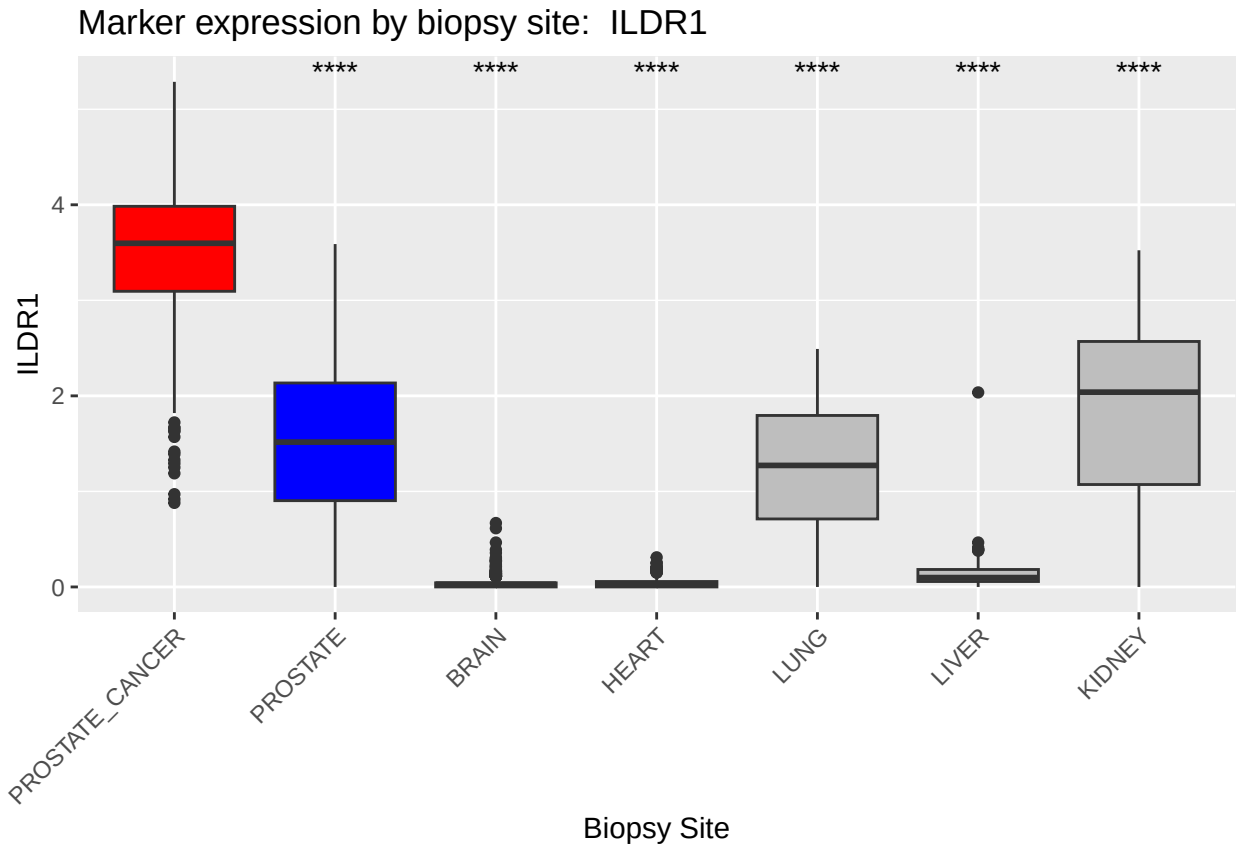
```
##  [1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
```

```
marker_expr=rbind(case_with_control_expr2,healthy_tissues_expr)

table(marker_expr$Sample)
```

```
##
##           BRAIN           HEART          KIDNEY           LIVER            LUNG
##            1148             376              28             110             288
##        PROSTATE PROSTATE_CANCER
##             100             494
```

```
sample_order <- c("PROSTATE_CANCER","PROSTATE", "BRAIN", "HEART", "LUNG","LIVER","KIDNEY")

marker_expr$Sample <- factor(marker_expr$Sample, levels = sample_order)

site_comparisons = list(c("PROSTATE_CANCER", "PROSTATE"), c("PROSTATE_CANCER", "BRAIN"),c("PROSTATE_CANC


# PLOT FIG. 2E with STATS

ggplot(marker_expr, aes(x = Sample, y = marker_expr[[5]], fill = Sample)) +
      geom_boxplot() +
      scale_fill_manual(values = c("red", "blue",rep("gray", length(sample_order) - 2)),guide = "none")
      labs(x = "Biopsy Site", y = names(marker_expr)[5], title = paste("Marker expression by biopsy site
      theme(axis.text.x = element_text(angle=45, hjust=1)) +
```

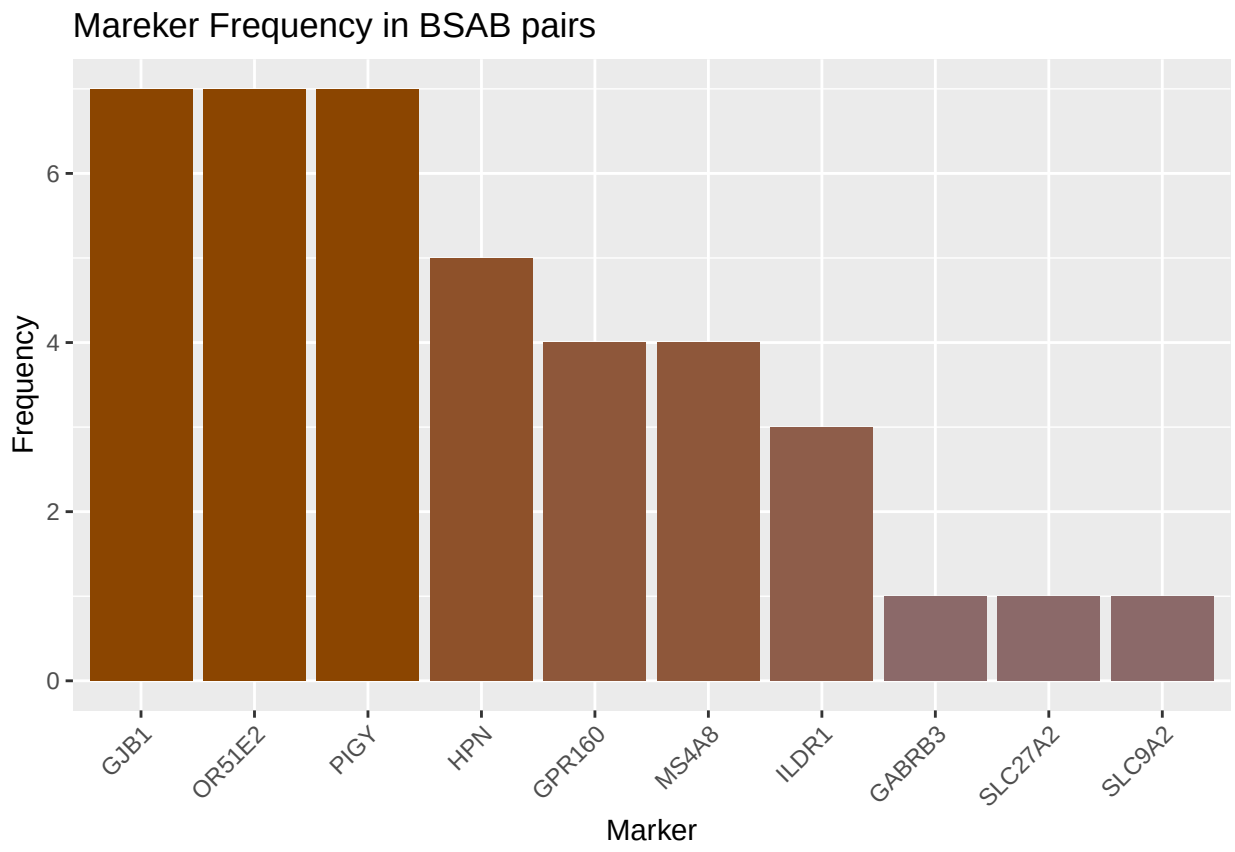
```
      #guides(fill = none)+
      stat_compare_means(method = "t.test", ref.group = "PROSTATE_CANCER", label="p.signif")
```




```
# Similar to above plot , this code will generate plots for all markers.
# You can specify the working directory where you would like to save the pdf.

pdf("~/Downloads/BSAB_PROSTATE_marker.pdf")
{
  for (i in 1:length(marker_list)) {
    gg <- ggplot(marker_expr, aes(x = Sample, y = marker_expr[[i]], fill = Sample)) +
      geom_boxplot() +
      scale_fill_manual(values = c("red", "blue",rep("gray", length(sample_order) - 2)),guide = "none")
      labs(x = "Biopsy Site", y = names(marker_expr)[i], title = paste("Marker expression by biopsy site
      theme(axis.text.x = element_text(angle=45, hjust=1)) +
      stat_compare_means(method = "t.test", ref.group = "PROSTATE_CANCER", label="p.signif")

     print(gg)

  }
}
```

```
# PLOT FIG.2C Marker Frequency plot

markers <- c(small_res_selective$antigen_1,small_res_selective$antigen_2)
```

```r
# Count the occurrences of each antigen in the combined vector
marker_counts <- table(markers)

# Convert the result to a data frame
marker_counts_df <- as.data.frame(marker_counts)
names(marker_counts_df) <- c("Marker", "Frequency")

marker_counts_df <- marker_counts_df[order(-marker_counts_df$Frequency), ]
marker_counts_df$Marker <- factor(marker_counts_df$Marker, levels = marker_counts_df$Marker)

proxy_var <- as.numeric(marker_counts_df$Frequency)


gg_freq<-ggplot(marker_counts_df, aes(x= Marker, y=Frequency, fill=proxy_var)) +
    geom_bar(stat="identity")+
    scale_y_continuous(breaks = seq(0, max(marker_counts_df$Frequency)+2, by = 2), limits = c(0, max(ma
    theme(axis.text.x = element_text(angle=45, hjust=1))+
    labs(x = "Marker", y = "Frequency", title = "Mareker Frequency in BSAB pairs") +
    scale_fill_gradient(low = "rosybrown4",high = "darkorange4", guide = "none")

print(gg_freq)
```

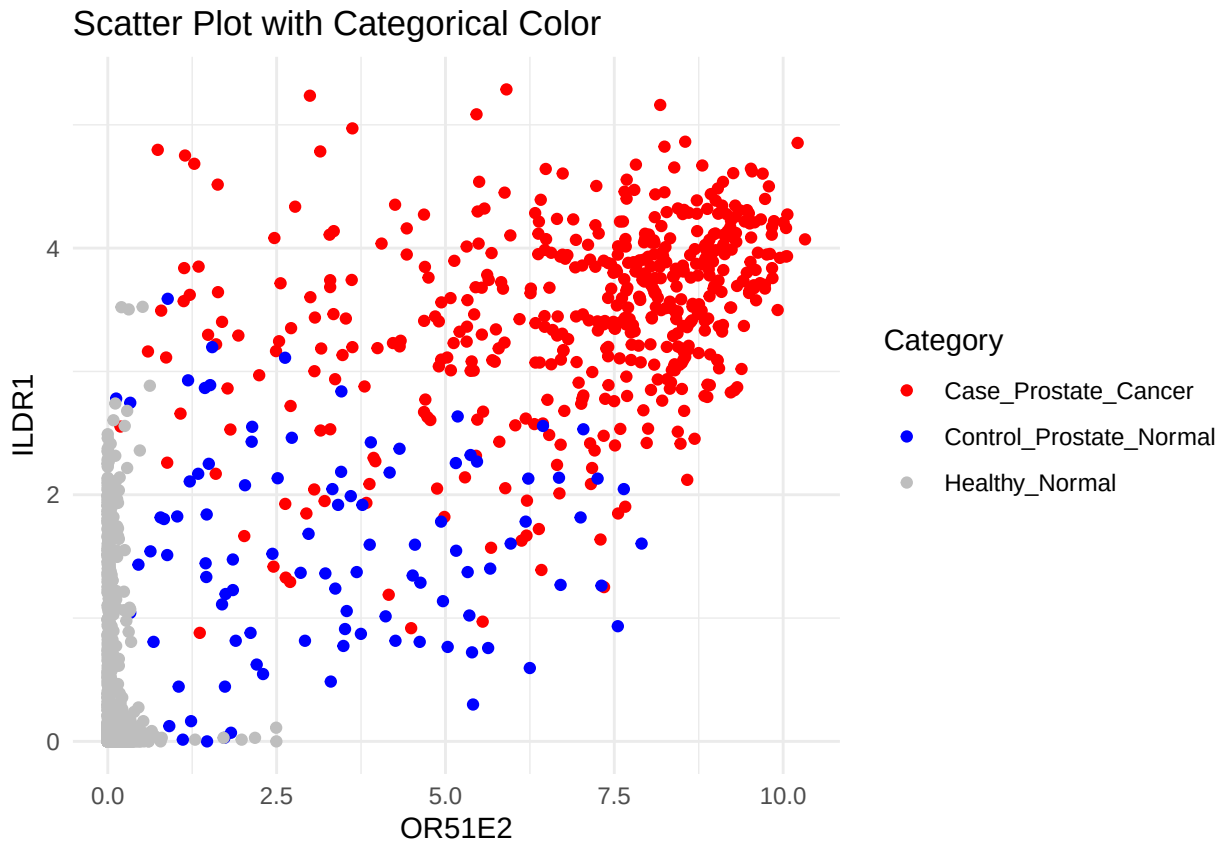



```r
# PLOT FIG.2B Marker Frequency plot

marker_expr$Category=NA
```

```r
unique(marker_expr$Sample)
```

```
## [1] PROSTATE_CANCER PROSTATE        HEART           BRAIN
## [5] LUNG            LIVER           KIDNEY
## Levels: PROSTATE_CANCER PROSTATE BRAIN HEART LUNG LIVER KIDNEY
```

```r
marker_expr$Category[marker_expr$Sample=="PROSTATE_CANCER"]="Case_Prostate_Cancer"
marker_expr$Category[marker_expr$Sample=="PROSTATE"]="Control_Prostate_Normal"
marker_expr$Category[marker_expr$Sample%in%c("HEART","BRAIN", "LUNG","LIVER","KIDNEY")]="Healthy_Normal"

ggplot(marker_expr, aes(x = OR51E2, y = ILDR1 , color = Category)) +
  geom_point() +
  labs(x = "OR51E2", y = "ILDR1", title = "Scatter Plot with Categorical Color") +
  scale_color_manual(values = c("Case_Prostate_Cancer" = "red", "Control_Prostate_Normal" = "blue", "Hea
  theme_minimal()
```




```r
# Save all important generated results

#save(list=c('case_expr','control_expr','case_with_control_expr','case_with_control_expr2','DE','DE1','
```