

bulk-RNAseq

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```
## Donor effect correction and analysis #####
## Overall comparison #####

d0 <- DGEList(counts) # Create DGEList object
d0 <- calcNormFactors(d0)
cutoff <- 10 # genes expressed in at least 10 samples to be included
drop <- which(apply(cpm(d0), 1, max) < cutoff)
d <- d0[-drop,]
dim(d) # number of genes left

## [1] 11374     84

snames <- colnames(counts) # Sample names

exposure <- substr(snames, 4, (nchar(snames)-1)) # for exposure groups
exposure <- as.factor(exposure)
exposure

## [1] CBS CBF PWS PWF PLS PLF CTR CBS CBF PWS PWF PLS PLF CTR CBS CBF PWS PWF PLS
## [20] PLF CTR CBS CBF PWS PWF PLS PLF CTR CBS CBF PWS PWF PLS PLF CTR CBS CBF PWS
## [39] PWF PLS PLF CTR CBS CBF PWS PWF PLS PLF CTR CBS CBF PWS PWF PLS PLF CTR CBS
## [58] CBF PWS PWF PLS PLF CTR CBS CBF PWS PWF PLS PLF CTR CBS CBF PWS PWF PLS PLF
## [77] CTR CBS CBF PWS PWF PLS PLF CTR
## Levels: CBF CBS CTR PLF PLS PWF PWS

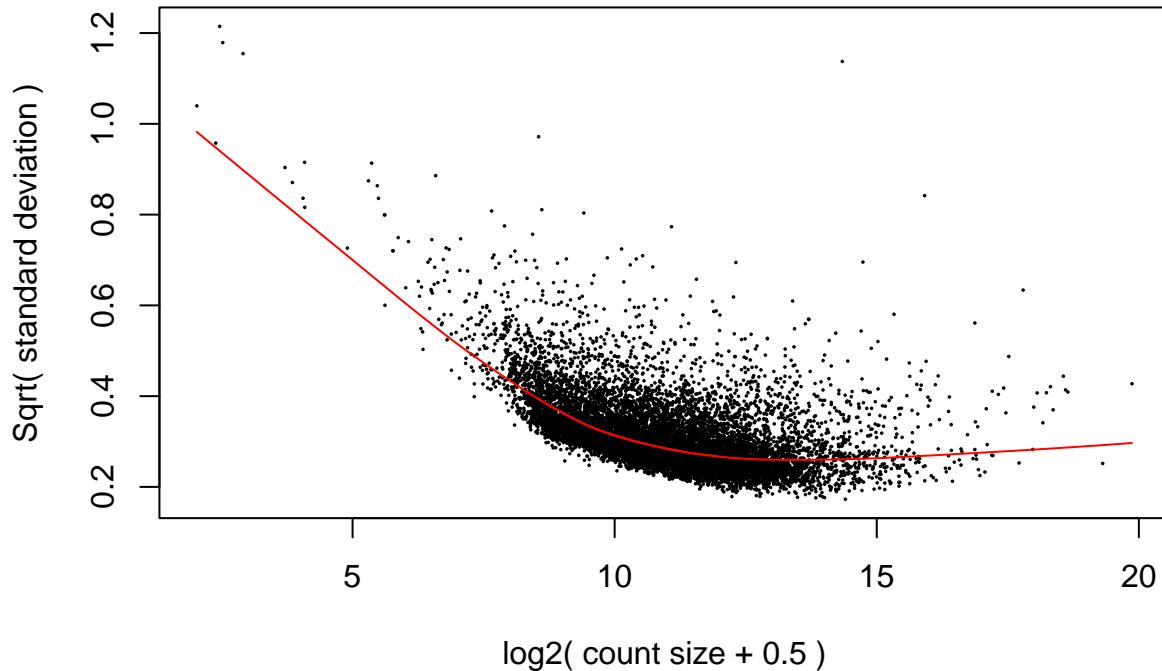
donor <- substr(snames, 1, (nchar(snames)-4)) # for donor based batch correction
batch <- as.factor(donor)
batch

## [1] NF1 NF1 NF1 NF1 NF1 NF1 NF1 NF2 NF2 NF2 NF2 NF2 NF3 NF3 NF3 NF3 NF3 NF3
## [20] NF3 NF3 NM1 NM1 NM1 NM1 NM1 NM1 NM1 NM2 NM2 NM2 NM2 NM2 NM3 NM3 NM3
## [39] NM3 NM3 NM3 SF1 SF1 SF1 SF1 SF1 SF2 SF2 SF2 SF2 SF2 SF2 SF3
## [58] SF3 SF3 SF3 SF3 SF3 SM1 SM1 SM1 SM1 SM1 SM1 SM2 SM2 SM2 SM2 SM2 SM2
## [77] SM2 SM3 SM3 SM3 SM3 SM3 SM3
## Levels: NF1 NF2 NF3 NM1 NM2 NM3 SF1 SF2 SF3 SM1 SM2 SM3

mm4 <- model.matrix(~0+exposure+batch)

y4 <- voom(d, mm4, plot = T)
```

voom: Mean–variance trend



```
colnames(mm4)
```

```
## [1] "exposureCBF" "exposureCBS" "exposureCTR" "exposurePLF" "exposurePLS"
## [6] "exposurePWF" "exposurePWS" "batchNF2"      "batchNF3"      "batchNM1"
## [11] "batchNM2"     "batchNM3"     "batchSF1"      "batchSF2"      "batchSF3"
## [16] "batchSM1"     "batchSM2"     "batchSM3"
```

```
fit4 <- lmFit(y4, mm4) # Fitting linear models in limma
```

```
head(coef(fit4))
```

	exposureCBF	exposureCBS	exposureCTR	exposurePLF	exposurePLS
## ENSG00000225630	3.697620	3.446782	3.628111	3.664299	3.612861
## ENSG00000237973	3.901321	3.684251	3.998707	4.008981	3.905990
## ENSG00000248527	7.306140	7.069484	7.388686	7.444703	7.354440
## ENSG00000228794	3.050411	3.033723	3.125936	3.066996	3.027666
## ENSG00000188976	6.360620	6.293310	6.330709	6.338577	6.279730
## ENSG00000187961	2.845102	2.811081	2.773978	2.781415	2.884708
	exposurePWF	exposurePWS	batchNF2	batchNF3	batchNM1
## ENSG00000225630	3.604670	3.680598	-0.03865659	0.72876535	0.67167071
## ENSG00000237973	3.875996	3.951327	0.48934509	0.32637702	0.35401991
## ENSG00000248527	7.301502	7.303130	0.61286730	0.50634463	0.75620339
## ENSG00000228794	3.055119	2.968585	0.08120150	0.22993662	0.26083341
## ENSG00000188976	6.453554	6.302150	-0.05184930	0.04073716	0.02015945
## ENSG00000187961	2.899971	2.836370	-0.05266860	-0.09454157	-0.24889394

```

##          batchNM2      batchNM3      batchSF1      batchSF2      batchSF3
## ENSG00000225630  0.59749883 -0.31028345  0.46645086 -0.52271901 -0.10739134
## ENSG00000237973 -0.09210945  0.36523068  0.42619308  0.29012936  0.82430014
## ENSG00000248527  0.32899689  0.33009829  0.72160485 -0.13047554  0.46209559
## ENSG00000228794  0.01579396  0.16625192 -0.01161010  0.32660041  0.27514590
## ENSG00000188976 -0.14408216  0.03283994 -0.08015085  0.08957051 -0.02648292
## ENSG00000187961 -0.04491268 -0.15662496 -0.09969143  0.33463467 -0.01079946
##          batchSM1      batchSM2      batchSM3
## ENSG00000225630 -0.15512373  0.68493648 -0.23802531
## ENSG00000237973 -0.10522468  0.56713121  0.16244740
## ENSG00000248527  0.23831248  1.11438948  0.25020613
## ENSG00000228794 -0.15244244  0.19871976  0.29251349
## ENSG00000188976  0.00432418  0.01866049 -0.08936964
## ENSG00000187961  0.31187416  0.27911658 -0.31237282

x <- colnames(coef(fit4))
length(x)

## [1] 18

x # to see the groups

## [1] "exposureCBF" "exposureCBS" "exposureCTR" "exposurePLF" "exposurePLS"
## [6] "exposurePWF" "exposurePWS" "batchNF2"      "batchNF3"      "batchNM1"
## [11] "batchNM2"     "batchNM3"     "batchSF1"      "batchSF2"      "batchSF3"
## [16] "batchSM1"     "batchSM2"     "batchSM3"

x <- x[1:7] # selecting levels representing the exposure groups
# to use in the "for" loop

a4 <- list() # list of both coding and non-coding transcripts
b4 <- list() # for storing analyzed data fro significantly altered genes
c4 <- list() # for getting the list of all genes
d4 <- list() # for storing ENSEMBL transcript names

for(i in 1:length(x)){if(x[i] != "exposureCTR"){
  difference <- paste(x[i],"-", "exposureCTR", sep="")
  contr <- makeContrasts(difference, levels = colnames(coef(fit4)))
  tmp <- contrasts.fit(fit4, contr)
  tmp <- eBayes(tmp)
  top.table <- topTable(tmp, sort.by = "P", n = Inf)
  l <- length(which(top.table$adj.P.Val <= 0.1 & abs(top.table$logFC) >= 0.5))
  exposure <- substr(x[i], nchar(x[i])-2, nchar(x[i]))
  print(paste("Number of transcripts changed in : ", exposure, l))
  silent=TRUE
  top.table <- as.data.frame(top.table)
  try(top.table$symbol <- mapIds(org.Hs.eg.db, keys = row.names(top.table),
                                 keytype = "ENSEMBL", column = "SYMBOL",
                                 multiVals="first")) #adding gene names
  top.table <- subset(top.table, top.table$symbol != 'NA')
  c4[[i]] <- top.table
  names(c4)[i] <- exposure
}

```

```

top.table<- top.table %>%
  mutate(direction = case_when(logFC > 0.5 ~ "up",
                               logFC < -0.5 ~ "down"))
top.table <- top.table[(which(top.table$adj.P.Val < 0.1 &
                                abs(top.table$logFC) > 0.5)),]
d4[[i]] = row.names(top.table)
names(d4)[i] <- exposure
rownames(top.table) <- NULL
#rownames(top.table) <- top.table$symbol
top.table <- top.table [(c(7,1,2,3,4,5,6,8))]
a4[[i]] = top.table$symbol
names(a4)[i] <- exposure
b4[[i]] <- top.table
names(b4)[i] <- exposure}
}

## [1] "Number of transcripts changed in : CBF 106"

## 'select()' returned 1:many mapping between keys and columns

## [1] "Number of transcripts changed in : CBS 39"

## 'select()' returned 1:many mapping between keys and columns

## [1] "Number of transcripts changed in : PLF 96"

## 'select()' returned 1:many mapping between keys and columns

## [1] "Number of transcripts changed in : PLS 16"

## 'select()' returned 1:many mapping between keys and columns

## [1] "Number of transcripts changed in : PWF 338"

## 'select()' returned 1:many mapping between keys and columns

## [1] "Number of transcripts changed in : PWS 7"

## 'select()' returned 1:many mapping between keys and columns

# removing the empty control group from the lists
a4[3] <- NULL
b4[3] <- NULL
c4[3] <- NULL
d4[3] <- NULL

```

FIGURE 1

```

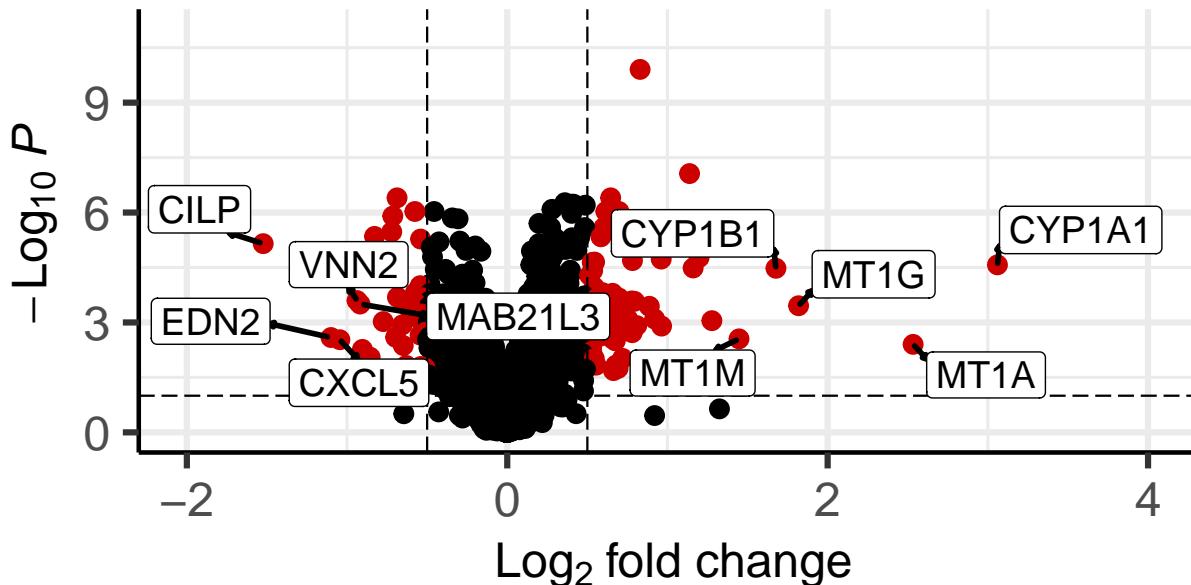
#printing volcano plot
res.df <- as.data.frame(c4[1]) # Cardboard Flaming
colnames(res.df) <- substr(colnames(res.df), 5, (nchar(colnames(res.df)))))

EnhancedVolcano(res.df,
  lab = res.df$symbol,
  x = 'logFC',
  y = 'adj.P.Val',
  title = 'Cardboard Flaming',
  xlim = c(-2.0, 4.0),
  ylim = c(0, -log10(10e-12)),
  pCutoff = 10e-2,
  pCutoffCol = 'adj.P.Val',
  FCcutoff = 0.5,
  boxedLabels = TRUE,
  parseLabels = TRUE,
  selectLab = c('CILP', 'EDN2', 'CXCL5', 'VNN2', 'MAB21L3',
               'CYP1A1', 'MT1A', 'MT1G', 'CYP1B1', 'MT1M'),
  col = c('black', 'black', 'black', 'red3'),
  pointSize = 3.0,
  labSize = 5.0,
  colAlpha = 1,
  legendPosition = 'none',
  legendLabSize = 12,
  legendIconSize = 5.0,
  drawConnectors = TRUE,
  widthConnectors = 1.0,
  colConnectors = 'black')

```

Cardboard Flaming

EnhancedVolcano



total = 11194 variables

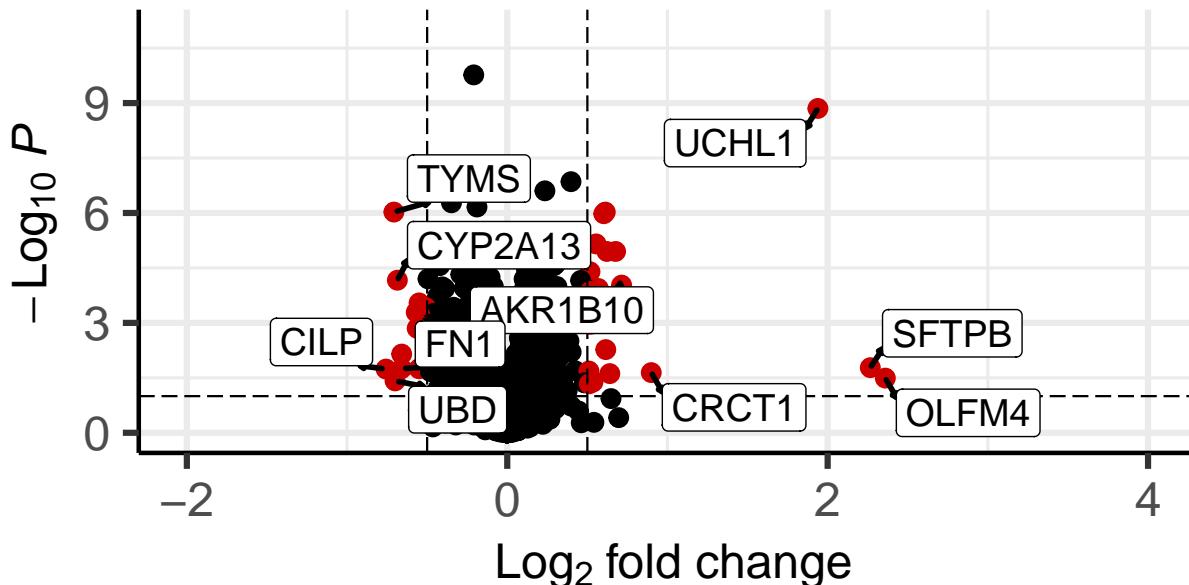
```
#printing volcano plot
res.df <- as.data.frame(c4[2]) # Cardboard Smoldering
colnames(res.df) <- substr(colnames(res.df), 5, (nchar(colnames(res.df)))))

EnhancedVolcano(res.df,
  lab = res.df$symbol,
  x = 'logFC',
  y = 'adj.P.Val',
  title = 'Cardboard Smoldering',
  xlim = c(-2.0, 4.0),
  ylim = c(0, -log10(10e-12)),
  pCutoff = 10e-2,
  pCutoffCol = 'adj.P.Val',
  FCcutoff = 0.5,
  boxedLabels = TRUE,
  parseLabels = TRUE,
  selectLab = c('CILP', 'TYMS', 'UBD', 'CYP2A13', 'FN1',
               'OLFM4', 'SFTPB', 'UCHL1', 'CRCT1', 'AKR1B10'),
  col = c('black', 'black', 'black', 'red3'),
  pointSize = 3.0,
  labSize = 5.0,
  colAlpha = 1,
  legendPosition = 'none',
  legendLabSize = 12,
  legendIconSize = 5.0,
  drawConnectors = TRUE,
```

```
widthConnectors = 1.0,
colConnectors = 'black')
```

Cardboard Smoldering

EnhancedVolcano



total = 11194 variables

```
#printing volcano plot
res.df <- as.data.frame(c4[5]) # Plywood Flaming
colnames(res.df) <- substr(colnames(res.df), 5, (nchar(colnames(res.df)))))

EnhancedVolcano(res.df,
  lab = res.df$symbol,
  x = 'logFC',
  y = 'adj.P.Val',
  title = 'Plywood Flaming',
  xlim = c(-2.0, 4.0),
  ylim = c(0, -log10(10e-20)),
  pCutoff = 10e-2,
  pCutoffCol = 'adj.P.Val',
  FCcutoff = 0.5,
  boxedLabels = TRUE,
  parseLabels = TRUE,
  selectLab = c('PTPRT', 'CILP', 'EDN2', 'ANPEP', 'SEC14L3',
               'CYP1A1', 'UCHL1', 'CYP1B1', 'AKR1B10', 'CYP1B1-AS1'),
  col = c('black', 'black', 'black', 'red3'),
  pointSize = 3.0,
  labSize = 5.0,
  colAlpha = 1,
```

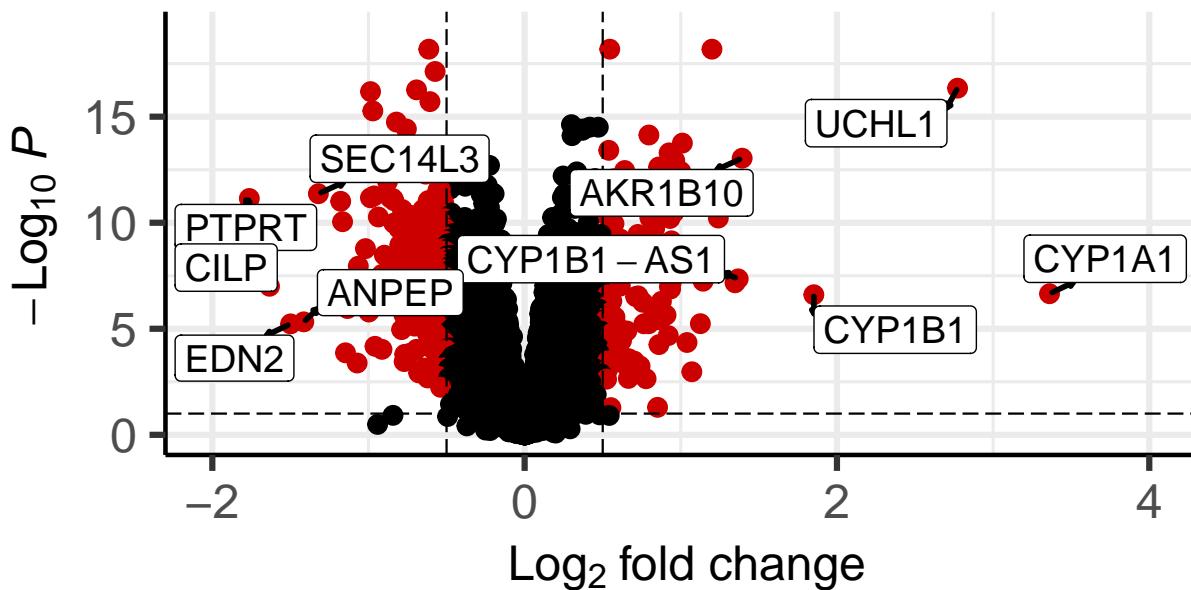
```

    legendPosition = 'none',
    legendLabSize = 12,
    legendIconSize = 5.0,
    drawConnectors = TRUE,
    widthConnectors = 1.0,
    colConnectors = 'black')

```

Plywood Flaming

EnhancedVolcano



total = 11194 variables

```

#printing volcano plot
res.df <- as.data.frame(c4[6]) # Plywood Smoldering
colnames(res.df) <- substr(colnames(res.df), 5, (nchar(colnames(res.df)))))

EnhancedVolcano(res.df,
  lab = res.df$symbol,
  x = 'logFC',
  y = 'adj.P.Val',
  title = 'Plywood Smoldering',
  xlim = c(-2.0, 4.0),
  ylim = c(0, -log10(10e-20)),
  pCutoff = 10e-2,
  pCutoffCol = 'adj.P.Val',
  FCcutoff = 0.5,
  boxedLabels = TRUE,
  parseLabels = TRUE,
  selectLab = c('STC2', 'CHAC1', 'CILP', 'DUSP2', 'UCHL1',
  'MUC5AC', 'HSPA6'),
  legendPosition = 'none',
  legendLabSize = 12,
  legendIconSize = 5.0,
  drawConnectors = TRUE,
  widthConnectors = 1.0,
  colConnectors = 'black')

```

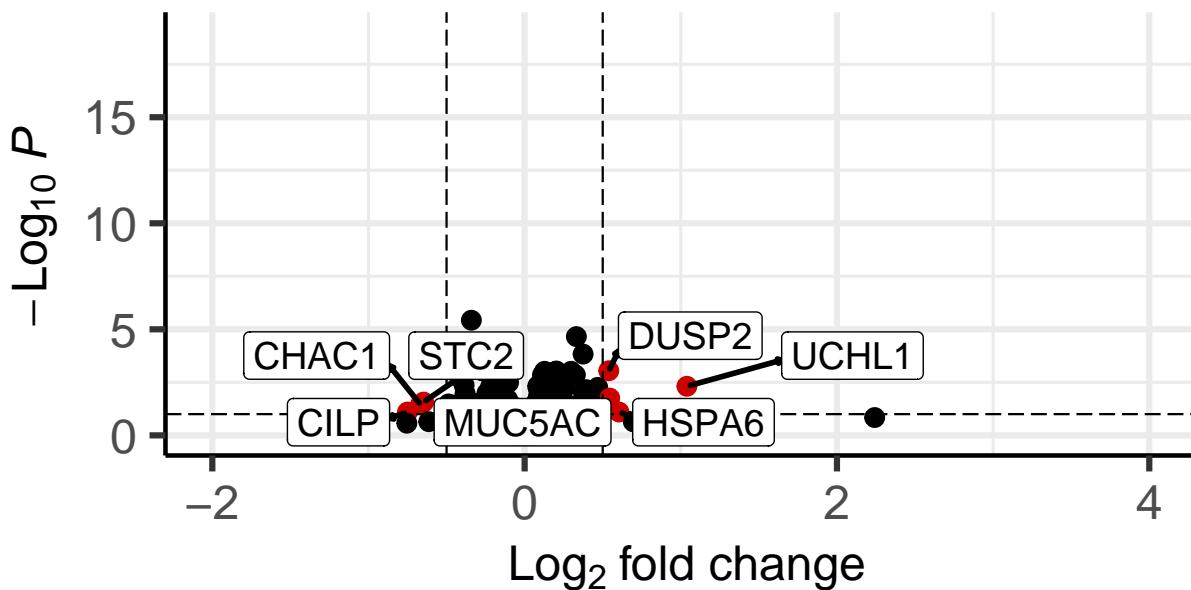
```

col = c('black', 'black', 'black', 'red3'),
pointSize = 3.0,
labSize = 5.0,
colAlpha = 1,
legendPosition = 'none',
legendLabSize = 12,
legendIconSize = 5.0,
drawConnectors = TRUE,
widthConnectors = 1.0,
colConnectors = 'black')

```

Plywood Smoldering

EnhancedVolcano



total = 11194 variables

```

#printing volcano plot
res.df <- as.data.frame(c4[3]) # Plastic Flaming
colnames(res.df) <- substr(colnames(res.df), 5, (nchar(colnames(res.df)))))

EnhancedVolcano(res.df,
                 lab = res.df$symbol,
                 x = 'logFC',
                 y = 'adj.P.Val',
                 title = 'Plastic Flaming',
                 xlim = c(-2.0, 4.0),
                 ylim = c(0, -log10(10e-12)),
                 pCutoff = 10e-2,
                 pCutoffCol = 'adj.P.Val',
                 FCcutoff = 0.5,

```

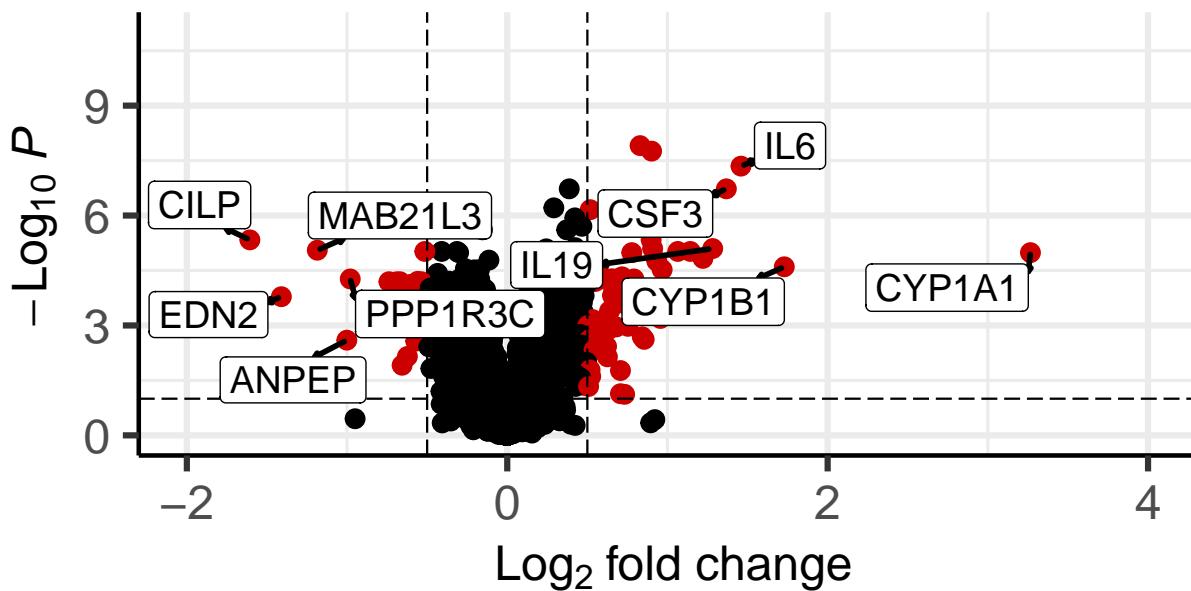
```

boxedLabels = TRUE,
parseLabels = TRUE,
selectLab = c('CILP', 'EDN2', 'MAB21L3', 'ANPEP', 'PPP1R3C',
             'CYP1A1', 'CYP1B1', 'IL6', 'CSF3', 'IL19'),
col = c('black', 'black', 'black', 'red3'),
pointSize = 3.0,
labSize = 5.0,
colAlpha = 1,
legendPosition = 'none',
legendLabSize = 12,
legendIconSize = 5.0,
drawConnectors = TRUE,
widthConnectors = 1.0,
colConnectors = 'black')

```

Plastic Flaming

EnhancedVolcano



total = 11194 variables

```

#printing volcano plot
res.df <- as.data.frame(c4[4]) # Plastic Smoldering
colnames(res.df) <- substr(colnames(res.df), 5, (nchar(colnames(res.df)))))

EnhancedVolcano(res.df,
                 lab = res.df$symbol,
                 x = 'logFC',
                 y = 'adj.P.Val',
                 title = 'Plastic Smoldering',
                 xlim = c(-2.0, 4.0),

```

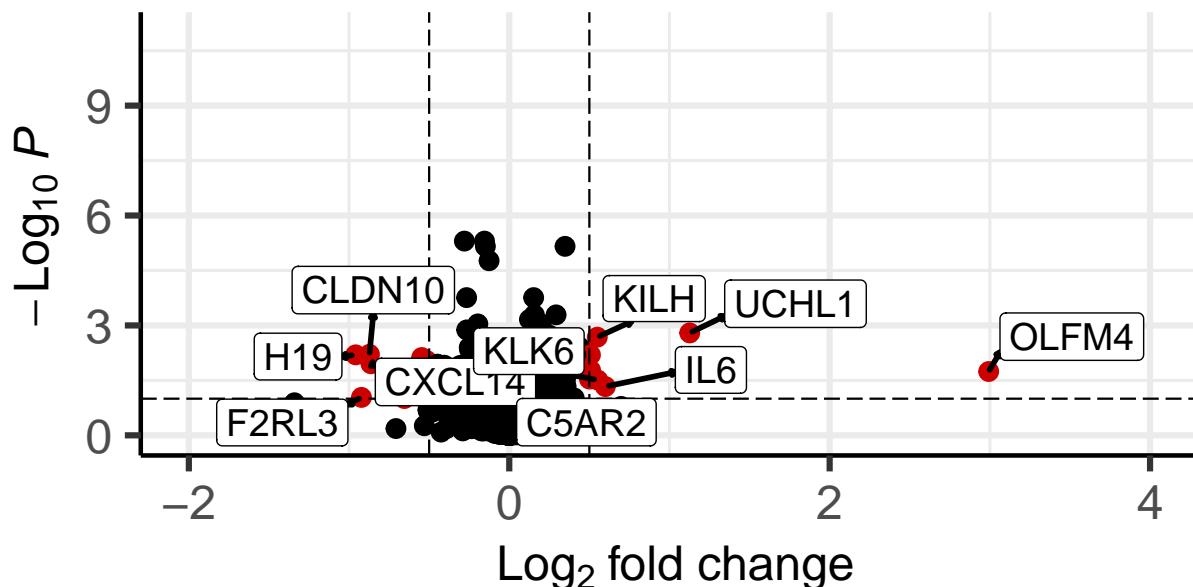
```

ylim = c(0, -log10(10e-12)),
pCutoff = 10e-2,
pCutoffCol = 'adj.P.Val',
FCcutoff = 0.5,
boxedLabels = TRUE,
parseLabels = TRUE,
selectLab = c( 'H19', 'F2RL3', 'CLDN10', 'CXCL14', 'C5AR2',
              'KILH', 'KLK6', 'IL6', 'UCHL1', 'OLFM4'),
col = c('black', 'black', 'black', 'red3'),
pointSize = 3.0,
labSize = 5.0,
colAlpha = 1,
legendPosition = 'none',
legendLabSize = 12,
legendIconSize = 5.0,
drawConnectors = TRUE,
widthConnectors = 1.0,
colConnectors = 'black')

```

Plastic Smoldering

EnhancedVolcano



total = 11194 variables

```

names(a4) <- str_replace_all(names(a4), c(CBF= "Cardboard\nFlaming",
                                            CBS= "Cardboard\nSmoldering",
                                            PLF= "Plastic\nFlaming",
                                            PLS= "Plastic\nSmoldering",
                                            PWF= "Plywood\nFlaming",
                                            PWS= "Plywood\nSmoldering"))

```

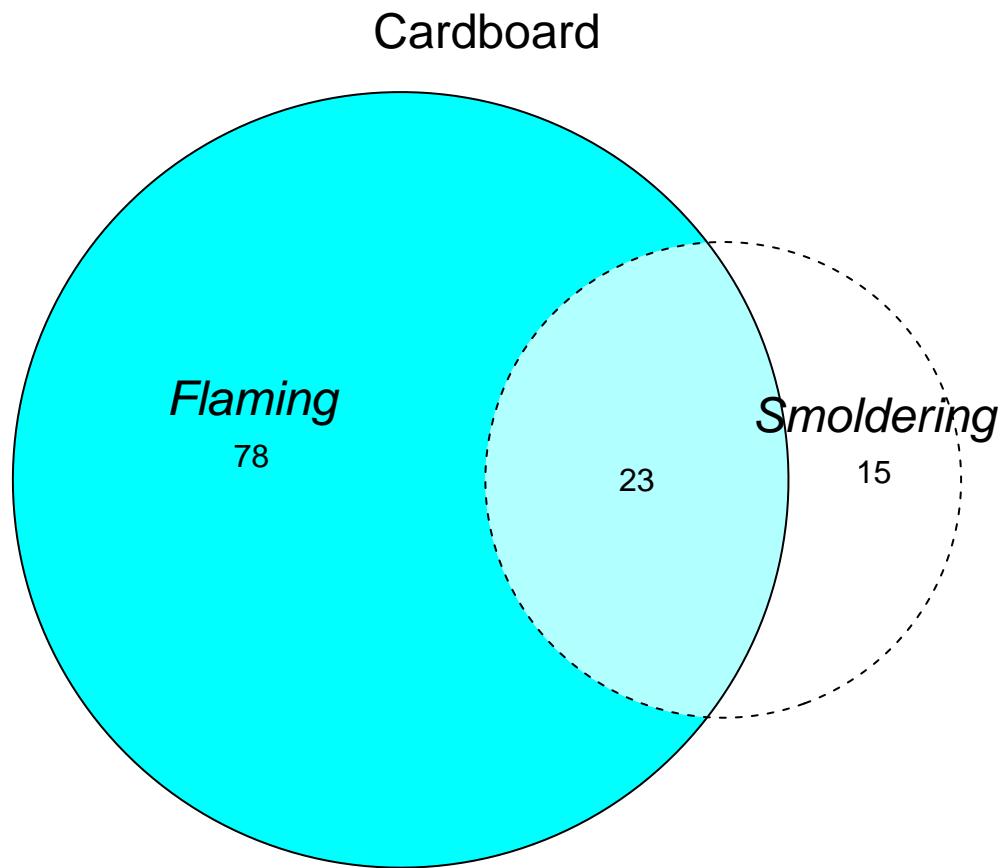
```

a4CB <- a4 %>%
  keep(str_detect(names(a4), "Cardboard"))
names(a4CB) <- sub('Cardboard\\n', '', names(a4CB))

EP_All_CB <- euler(a4CB, shape = "ellipse") # Euler plot

plot(EP_All_CB,
  quantities = TRUE, cex = 5,
  main = "Cardboard",
  lty = 1:3,
  labels = list(font = 3, cex = 1.5),
  fills = c("cyan", "white"))

```



```

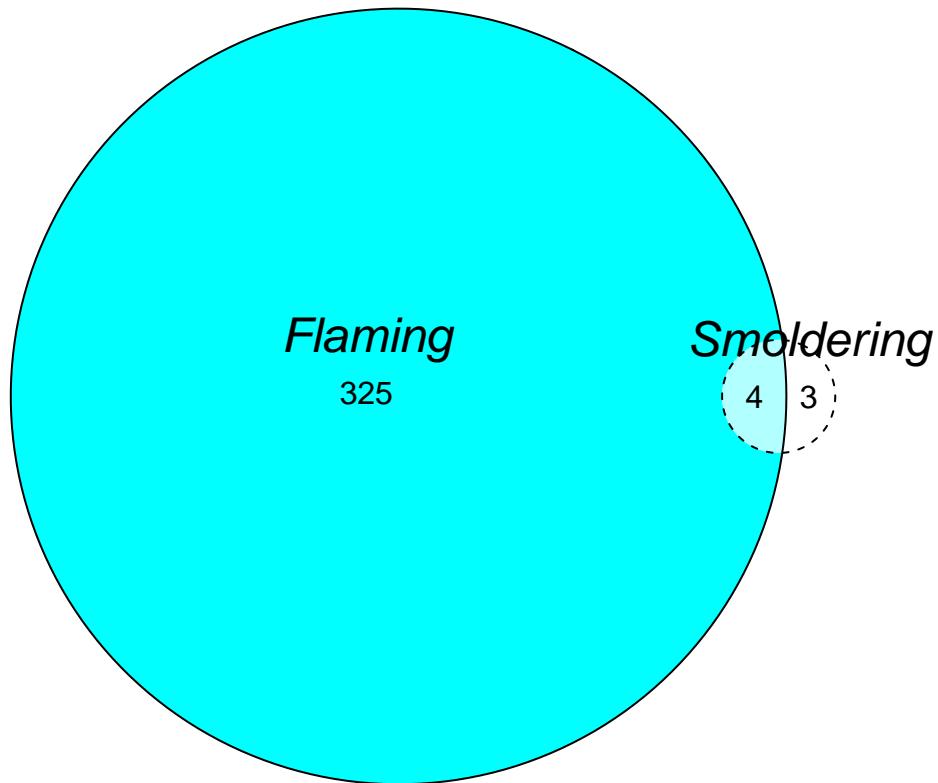
a4PW <- a4 %>%
  keep(str_detect(names(a4), "Plywood"))
names(a4PW) <- sub('Plywood\\n', '', names(a4PW))

EP_All_PW <- euler(a4PW, shape = "ellipse") # Euler plot

plot(EP_All_PW,
  quantities = TRUE, cex = 5,
  main = "Plywood",
  lty = 1:3,
  labels = list(font = 3, cex = 1.5),
  fills = c("cyan", "white"))

```

Plywood



```
a4PL <- a4 %>%
  keep(str_detect(names(a4), "Plastic"))
names(a4PL) <- sub('Plastic\\n', '', names(a4PL))

EP_All_PL <- euler(a4PL, shape = "ellipse") # Euler plot

plot(EP_All_PL,
      quantities = TRUE, cex = 5,
      main = "Plastic",
      lty = 1:3,
      labels = list(font = 3, cex = 1.5),
      fills = c("cyan", "white"))
```

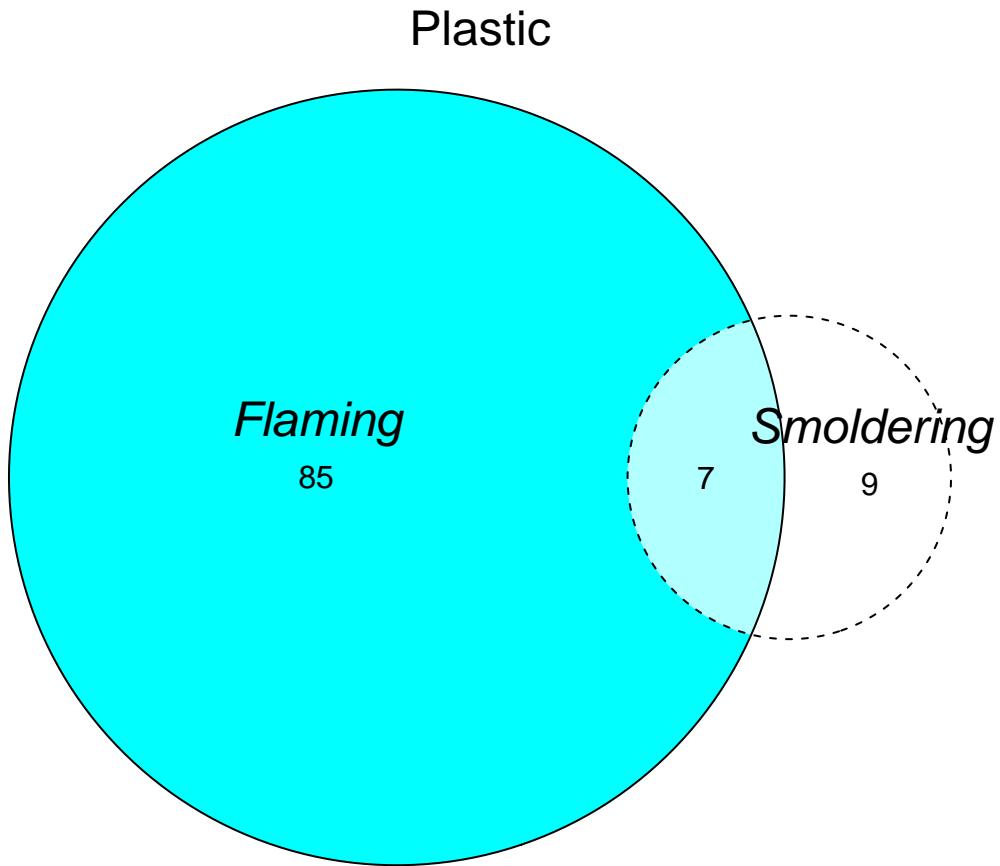
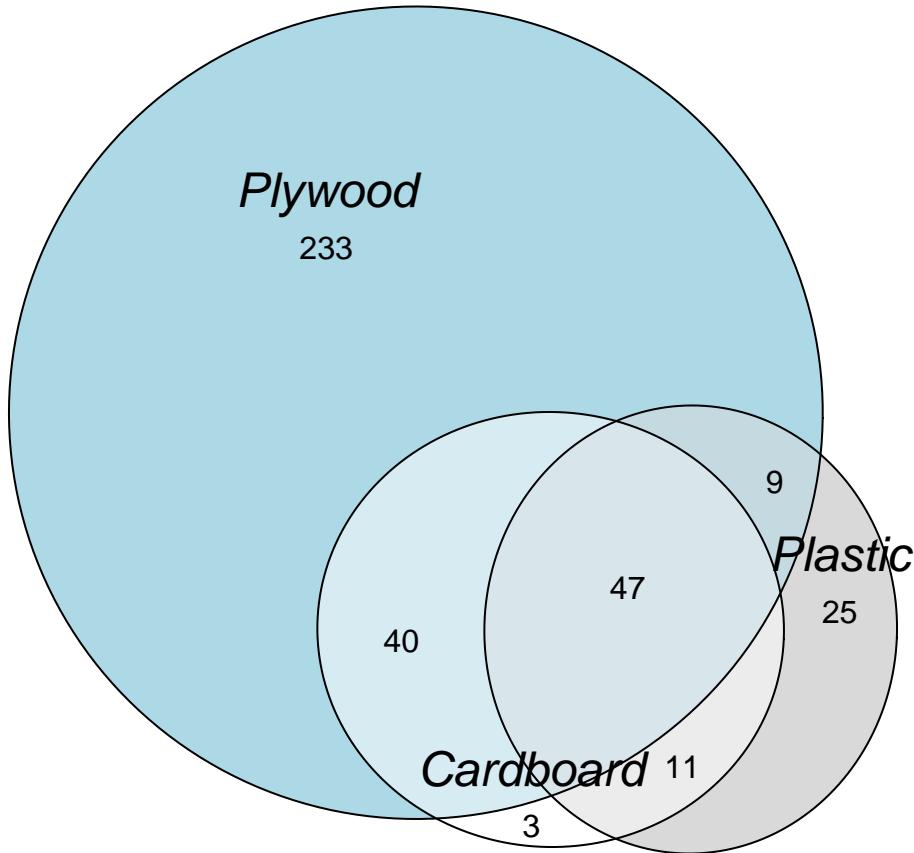


FIGURE 2

```
a4F <- a4 %>%
  keep(str_detect(names(a4), "Flaming"))
names(a4F) <- sub('\nFlaming', '', names(a4F))

EP_All_F <- euler(a4F, shape = "ellipse") # Euler plot -flaming
plot(EP_All_F, quantities = TRUE, cex = 5, labels = list(font = 3, cex = 1.5))
```



```

names(d4) <- str_replace_all(names(d4), c(CBF= "Cardboard Flaming",
                                         CBS= "Cardboard Smoldering",
                                         PLF= "Plastic Flaming",
                                         PLS= "Plastic Smoldering",
                                         PWF= "Plywood Flaming",
                                         PWS= "Plywood Smoldering"))

d4F <- d4 %>%
  keep(str_detect(names(d4), "Flaming"))
names(d4F) <- sub('\nFlaming', '', names(d4F))

z <- process_region_data(Venn(d4F))
z <- as.data.frame(z)

counts_Fg <- counts %>% select(ends_with("CTRL") |
  ends_with("CBFh") |
  ends_with("PLFh") |
  ends_with("PwFh")) # selecting flaming samples

#-----#
# Control and flaming

counts_Fg_c <- subset(counts_Fg, rownames(counts_Fg) %in% c(unlist(z[7,3])))

counts_Fg_c <- counts_Fg_c %>% rownames_to_column(var = "transcripts")

```

```

counts_Fg_c <- counts_Fg_c %>% pivot_longer(cols = -1, names_to = "SampleID", values_to = "count")
counts_Fg_c$SampleID <- substr(counts_Fg_c$SampleID, 4, nchar(counts_Fg_c$SampleID))

counts_Fg_c$SampleID <- str_replace_all(counts_Fg_c$SampleID, c(CBFh = "Cardboard",
                                                               PLFh = "Plastic",
                                                               PWFh = "Plywood",
                                                               CTRL = "Control"))

colnames(counts_Fg_c)

## [1] "transcripts" "SampleID"      "count"

counts_ALL <- counts_Fg_c %>%
  group_by(transcripts, SampleID) %>%
  summarise(across(everything(), sum))

## `summarise()` has grouped output by 'transcripts'. You can override using the
## '.groups' argument.

counts_ALL <- counts_ALL %>% pivot_wider(names_from = "SampleID", values_from = "count") %>%
  column_to_rownames(var = "transcripts")

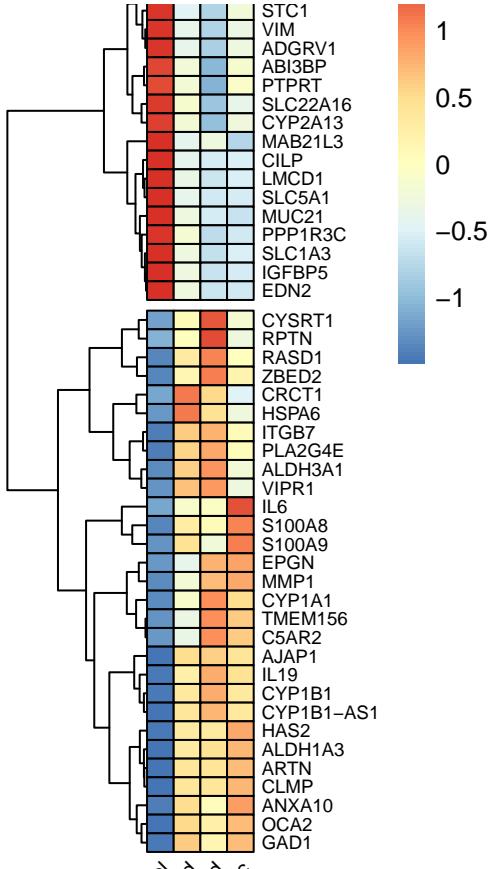
counts_ALL <- counts_ALL %>% relocate("Control", "Cardboard", "Plywood", "Plastic" )
counts_ALL$gene <- mapIds(org.Hs.eg.db, keys = row.names(counts_ALL),
                         keytype = "ENSEMBL", column = "SYMBOL",
                         multiVals="first") #adding gene names

## 'select()' returned 1:1 mapping between keys and columns

counts_ALL <- subset(counts_ALL, counts_ALL$gene != 'NA')
rownames(counts_ALL) <- NULL
rownames(counts_ALL) <- counts_ALL$gene
counts_ALL <- counts_ALL[,1:(ncol(counts_ALL)-1)]

temp3 <- as.matrix(counts_ALL)
pheatmap(temp3,
         color = colorRampPalette(rev(brewer.pal(n = 7, name ="RdYlBu")))(100),
         # sets color scheme
         display_numbers = FALSE,
         number_color = "black",
         fontsize_number = 5,
         main = "Flaming",
         angle_col = c("45"), # makes column labels horizontal
         cellwidth = 10, # sets dimensions of cells
         cellheight = 7, # sets dimensions of cells
         border_color = "black", # adds black border around cells
         treeheight_col = 5, # sets dims of trees
         fontsize_row = 7, # sets dims of trees
         scale = 'row', # scales data by row
         fontsize_col = 8, # sets font size for column labels
         cutree_rows = 2, # indicates how many clusters to show
         cluster_cols = FALSE,
         cluster_rows = TRUE)

```



```
#####
# GSEA and GO analyses
#####

OverL <- process_region_data(Venn(d4F)) #collecting intersect values

OverL$item[7] # viewing 47 transcripts commonly affected by all flaming condensates
```

```
## [[1]]
## [1] "ENSG00000139626" "ENSG00000164199" "ENSG00000100170" "ENSG00000159167"
## [5] "ENSG00000004809" "ENSG00000138615" "ENSG00000232973" "ENSG00000108602"
## [9] "ENSG00000140465" "ENSG00000142224" "ENSG00000138061" "ENSG00000128683"
## [13] "ENSG00000071282" "ENSG00000163220" "ENSG00000188089" "ENSG00000026025"
## [17] "ENSG00000114812" "ENSG00000177494" "ENSG00000173212" "ENSG00000143546"
## [21] "ENSG00000117407" "ENSG00000154175" "ENSG00000197838" "ENSG00000115461"
## [25] "ENSG00000170961" "ENSG00000108551" "ENSG00000169509" "ENSG00000196090"
## [29] "ENSG00000136244" "ENSG00000104044" "ENSG00000166250" "ENSG00000184254"
## [33] "ENSG00000079215" "ENSG00000173110" "ENSG00000197191" "ENSG00000109511"
## [37] "ENSG00000119938" "ENSG00000127129" "ENSG00000215853" "ENSG00000121895"
## [41] "ENSG00000204544" "ENSG00000166825" "ENSG00000182585" "ENSG00000134830"
## [45] "ENSG00000166828" "ENSG00000196581" "ENSG00000196611"
```

```
#-----#
```

```
#GO over-representation analysis
```

```

geneOL <- unlist(OverL$item[7])
enGOOL <- enrichGO(gene      = geneOL,
                     OrgDb       = org.Hs.eg.db,
                     keyType     = 'ENSEMBL',
                     ont         = "ALL",
                     pAdjustMethod = "BH",
                     pvalueCutoff  = 0.05,
                     qvalueCutoff   = 0.1)
head(enGOOL)

##          ONTOLOGY      ID           Description GeneRatio BgRatio
## GO:0009404    BP GO:0009404    toxin metabolic process 3/41 14/21261
## GO:0070486    BP GO:0070486    leukocyte aggregation 3/41 14/21261
## GO:0019373    BP GO:0019373    epoxigenase P450 pathway 3/41 18/21261
## GO:0014002    BP GO:0014002    astrocyte development 4/41 60/21261
## GO:0019748    BP GO:0019748    secondary metabolic process 4/41 66/21261
## GO:0050900    BP GO:0050900    leukocyte migration 7/41 463/21261
##          pvalue    p.adjust    qvalue
## GO:0009404 2.387331e-06 0.001426431 0.001060478
## GO:0070486 2.387331e-06 0.001426431 0.001060478
## GO:0019373 5.323196e-06 0.001603287 0.001191961
## GO:0014002 5.366651e-06 0.001603287 0.001191961
## GO:0019748 7.865896e-06 0.001879949 0.001397646
## GO:0050900 2.629613e-05 0.005237313 0.003893673
##
##          Count
## GO:0009404 3
## GO:0070486 3
## GO:0019373 3
## GO:0014002 4
## GO:0019748 4
## GO:0050900 7

#barplot
barplot(enGOOL, showCategory=10, font = 18, title = "47 -common genes")+
  scale_x_continuous(breaks = seq(0, 10, by = 2), limits=c(0,10))+
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))

```

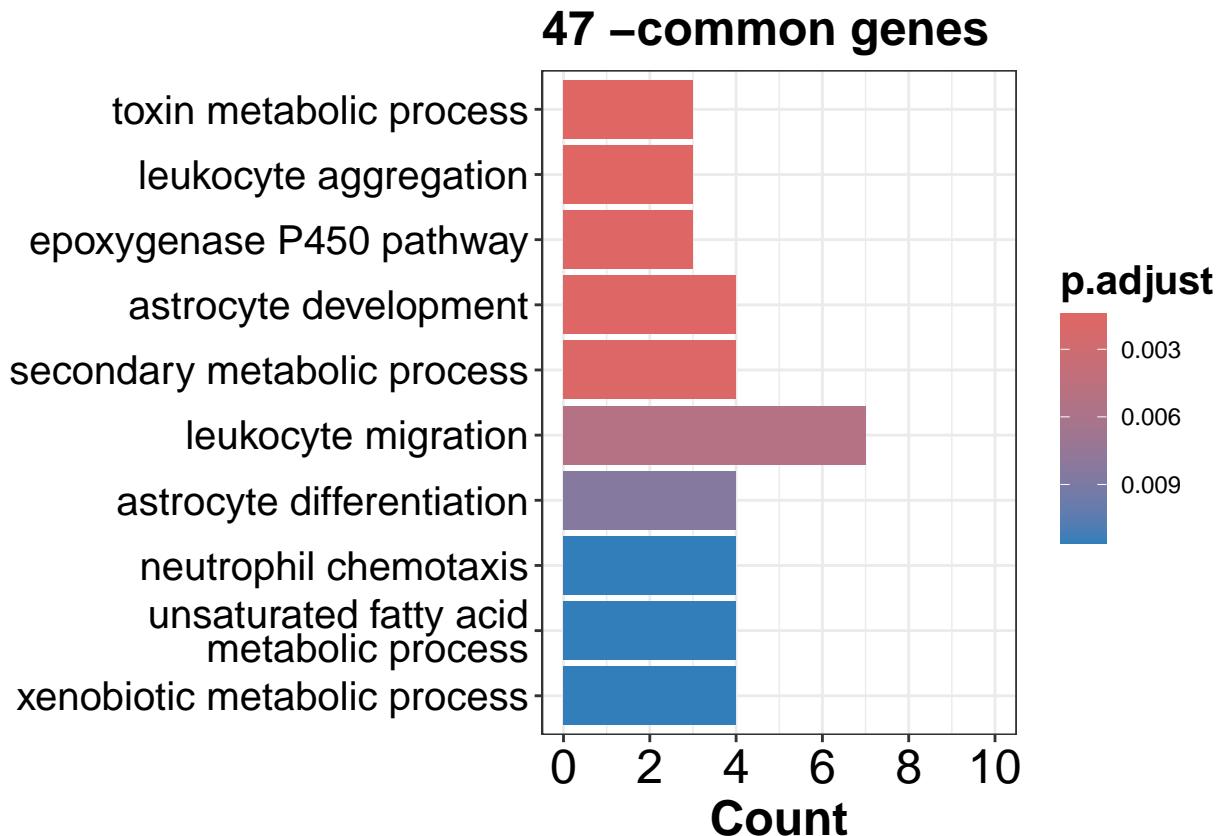


FIGURE 3

```
#####
# Reading in counts data

Gdata <- c4

#####
#-----#
# Cardboard exposure group -Flaming

Gdata$CBF <- Gdata$CBF %>% mutate(ProbeID = rownames(Gdata$CBF))

GdataCBf <- Gdata$CBF #Cardboard
GdataCBf <- select(GdataCBf, ProbeID, logFC)
rownames(GdataCBf) <- NULL

#making ranked gene list
genelist_GdataCBf = GdataCBf[,2] #numeric vector
names(genelist_GdataCBf) = as.character(GdataCBf[,1]) #named vector
genelist_GdataCBf = sort(genelist_GdataCBf, decreasing = TRUE) #must sort in descending order

#Performing GSEA analysis
#Gene Ontology (GO)
gseGO_CBF_ALL <- gseGO(geneList=genelist_GdataCBf,
                           ont ="ALL",
```

```

keyType = "ENSEMBL",
minGSSize = 10, #min size of gene sets for analysis
maxGSSize = 500, #max size of gene sets for analysis
pvalueCutoff = 0.05,
eps = 0,
verbose = TRUE,
OrgDb = organism,
pAdjustMethod = "BH") #Bbenjamini Hochberg adjustment

```

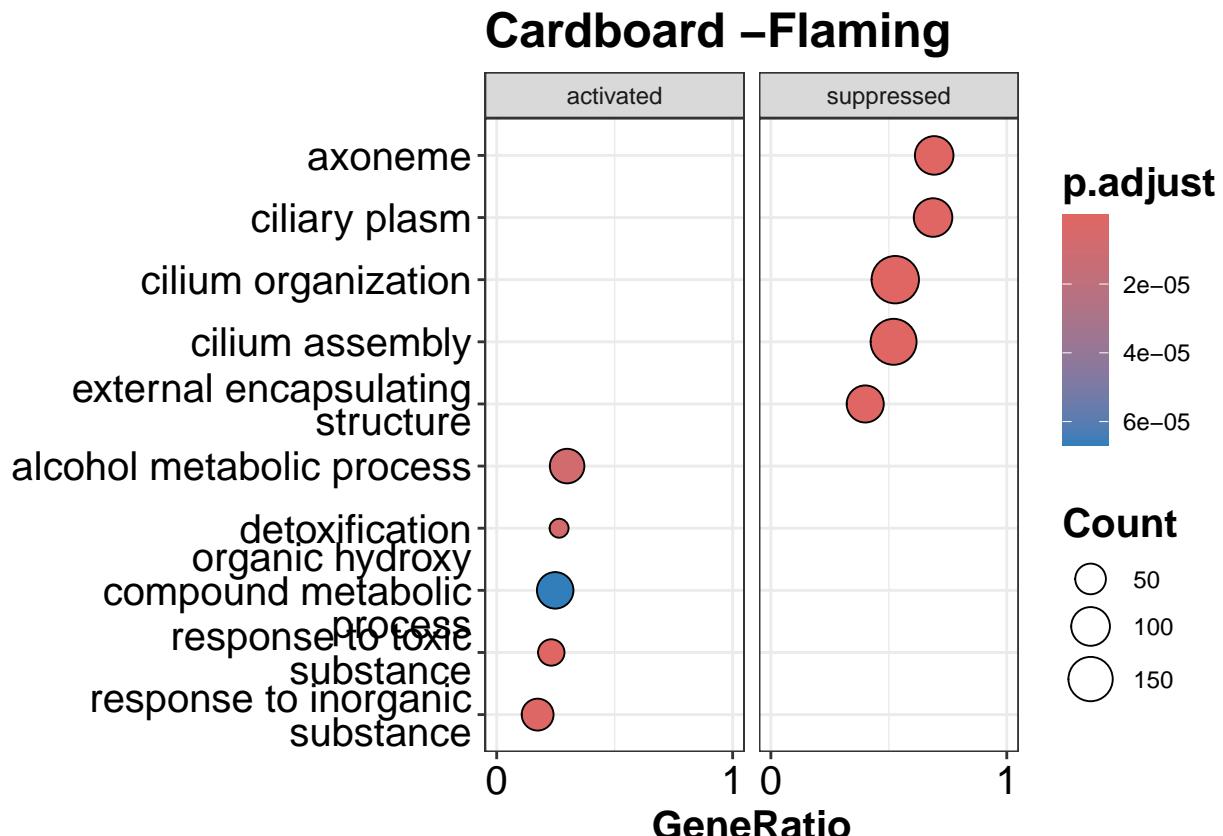
```
## preparing geneSet collections...
```

```
## GSEA analysis...
```

```
## leading edge analysis...
```

```
## done...
```

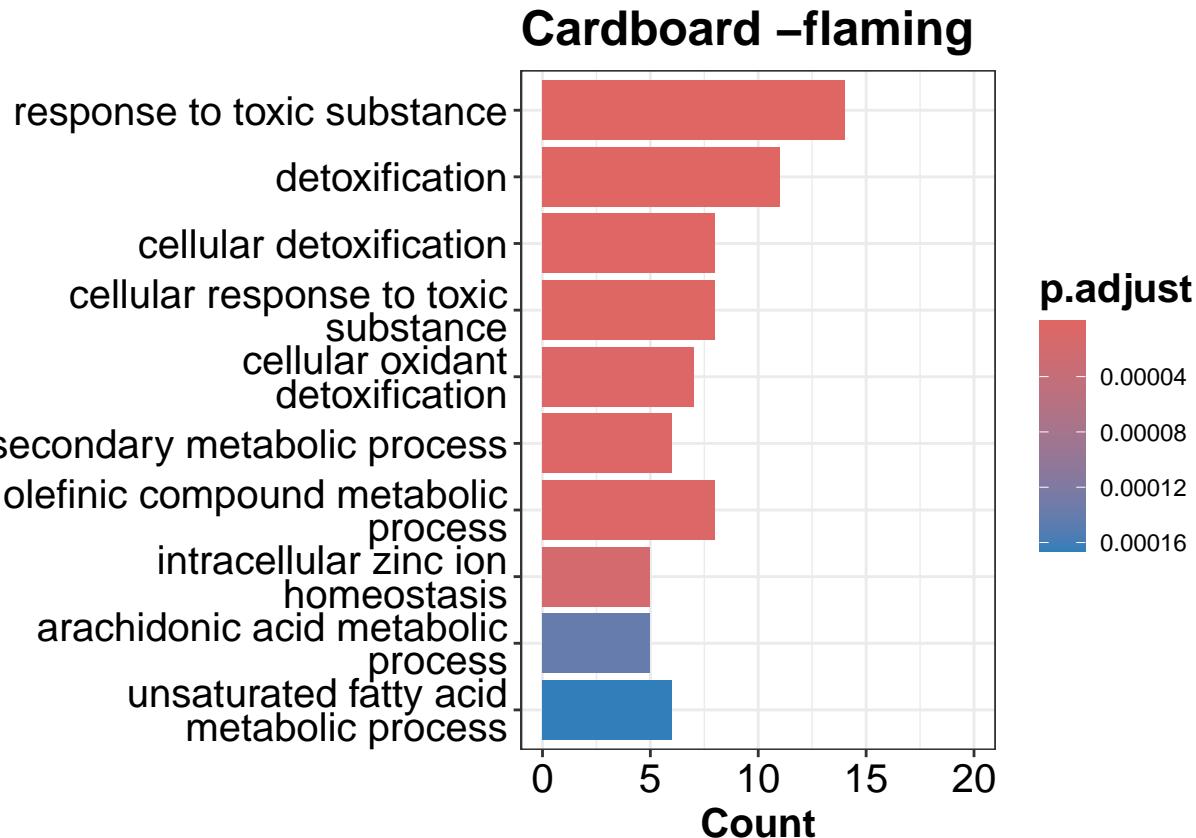
```
#dotplot
enrichplot::dotplot(gseGO_CBF_ALL, split=".sign", title = "Cardboard -Flaming",
                     showCategory = 5, font.size = 15,
                     label_format = 25) + facet_grid(.~.sign) +
  scale_x_continuous(breaks = seq(0, 1, by = 1), limits=c(0,1))+
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))
```



```
#-----#
#GO over-representation analysis
geneCBf <- names(genelist_GdataCBf[genelist_GdataCBf[] > 0.5]) # logFC > 0.5
enGOCBf <- enrichGO(gene      = geneCBf,
                      OrgDb     = org.Hs.eg.db,
                      keyType   = 'ENSEMBL',
                      ont       = "ALL",
                      pAdjustMethod = "BH",
                      pvalueCutoff = 0.05,
                      qvalueCutoff = 0.1)
head(enGOCBf)
```

##	ONTOLOGY	ID	Description	GeneRatio
## GO:0009636	BP	GO:0009636	response to toxic substance	14/59
## GO:0098754	BP	GO:0098754	detoxification	11/59
## GO:1990748	BP	GO:1990748	cellular detoxification	8/59
## GO:0097237	BP	GO:0097237	cellular response to toxic substance	8/59
## GO:0098869	BP	GO:0098869	cellular oxidant detoxification	7/59
## GO:0019748	BP	GO:0019748	secondary metabolic process	6/59
	BgRatio	pvalue	p.adjust	qvalue
## GO:0009636	294/21261	5.224242e-14	7.115418e-11	5.581690e-11
## GO:0098754	173/21261	1.499863e-12	1.021406e-09	8.012424e-10
## GO:1990748	132/21261	3.029243e-09	1.375276e-06	1.078836e-06
## GO:0097237	149/21261	7.894756e-09	2.688164e-06	2.108731e-06
## GO:0098869	112/21261	2.535123e-08	6.376023e-06	5.001672e-06
## GO:0019748	66/21261	2.808821e-08	6.376023e-06	5.001672e-06
	Count			
## GO:0009636	14			
## GO:0098754	11			
## GO:1990748	8			
## GO:0097237	8			
## GO:0098869	7			
## GO:0019748	6			

```
#barplot
barplot(enGOCBf, showCategory=10, font = 15, title = "Cardboard -flaming")+
  scale_x_continuous(breaks = seq(0, 20, by = 5), limits=c(0,20))+
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))
```



```
#####
#-----#
# Plywood exposure group -Flaming

Gdata$PWF <- Gdata$PWF %>% mutate(ProbeID = rownames(Gdata$PWF))

GdataPwf <- Gdata$PWF #Plywood

GdataPwf <- select(GdataPwf, ProbeID, logFC)
rownames(GdataPwf) <- NULL

#making ranked gene list
genelist_GdataPwf = GdataPwf[,2] #numeric vector
names(genelist_GdataPwf) = as.character(GdataPwf[,1]) #named vector
genelist_GdataPwf = sort(genelist_GdataPwf, decreasing = TRUE) #must sort in descending order

#Performing GSEA analysis
#Gene Ontology (GO)
gseGO_Pwf_ALL <- gseGO(geneList=genelist_GdataPwf,
                           ont ="ALL",
                           keyType = "ENSEMBL",
                           minGSSize = 10, #min size of gene sets for analysis
                           maxGSSize = 500, #max size of gene sets for analysis
                           pvalueCutoff = 0.05,
                           eps=0,
                           verbose = TRUE,
```

```

    OrgDb = organism,
    pAdjustMethod = "BH") #Benjamini Hochberg adjustment

```

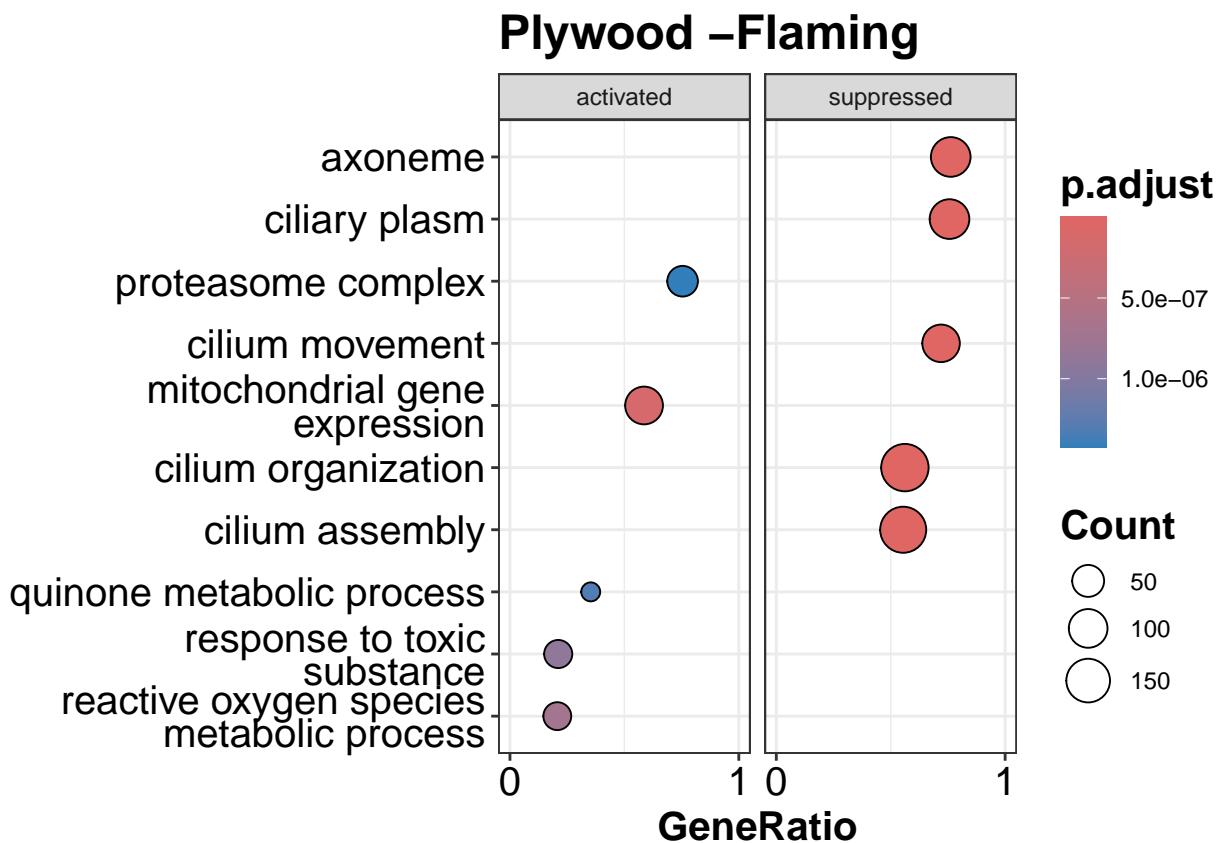
```
## preparing geneSet collections...
```

```
## GSEA analysis...
```

```
## leading edge analysis...
```

```
## done...
```

```
#dotplot
enrichplot::dotplot(gseGO_PWf_ALL, split=".sign", title = "Plywood -Flaming",
                     showCategory = 5, font.size = 15,
                     label_format = 25) + facet_grid(.~.sign) +
  scale_x_continuous(breaks = seq(0, 1, by = 1), limits=c(0,1)) +
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))
```



```
#-----#
```

```
#GO over-representation analysis
```

```
genePWf <- names(genelist_GdataPWf[genelist_GdataPWf[] > 0.5]) # logFC > 0.5
```

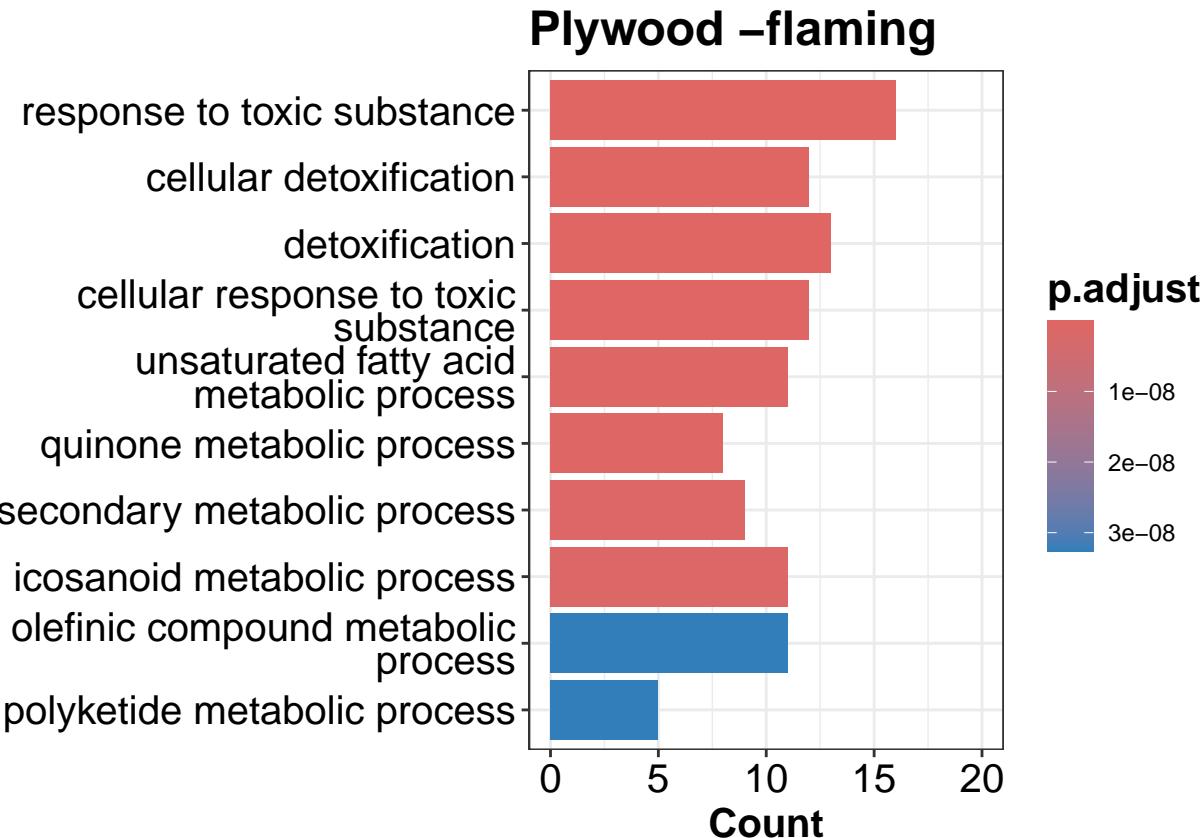
```

enGOPWf <- enrichGO(gene      = genePWf,
                     OrgDb     = org.Hs.eg.db,
                     keyType   = 'ENSEMBL',
                     ont       = "ALL",
                     pAdjustMethod = "BH",
                     pvalueCutoff  = 0.05,
                     qvalueCutoff  = 0.1)
head(enGOPWf)

##          ONTOLOGY      ID             Description
## GO:0009636    BP GO:0009636 response to toxic substance
## GO:1990748    BP GO:1990748 cellular detoxification
## GO:0098754    BP GO:0098754 detoxification
## GO:0097237    BP GO:0097237 cellular response to toxic substance
## GO:0033559    BP GO:0033559 unsaturated fatty acid metabolic process
## GO:1901661    BP GO:1901661 quinone metabolic process
##          GeneRatio    BgRatio      pvalue    p.adjust    qvalue
## GO:0009636    16/85 294/21261 3.969531e-14 6.903014e-11 5.519737e-11
## GO:1990748    12/85 132/21261 1.765488e-13 1.119805e-10 8.954102e-11
## GO:0098754    13/85 173/21261 1.931808e-13 1.119805e-10 8.954102e-11
## GO:0097237    12/85 149/21261 7.601711e-13 3.304844e-10 2.642595e-10
## GO:0033559    11/85 124/21261 2.506343e-12 7.508479e-10 6.003874e-10
## GO:1901661    8/85  39/21261 2.590619e-12 7.508479e-10 6.003874e-10
##
## GO:0009636 ENSG00000140465/ENSG00000138061/ENSG00000198074/ENSG00000176153/ENSG00000181019/ENSG00000
## GO:1990748                                         ENSG00000198074/ENSG00000
## GO:0098754                                         ENSG00000198074/ENSG00000176153/ENSG00000
## GO:0097237                                         ENSG00000198074/ENSG00000
## GO:0033559                                         ENSG00000198074/ENSG00000
## GO:1901661                                         ENSG00000
##          Count
## GO:0009636    16
## GO:1990748    12
## GO:0098754    13
## GO:0097237    12
## GO:0033559    11
## GO:1901661    8

#barplot
barplot(enGOPWf, showCategory=10, font = 15, title = "Plywood -flaming")+
  scale_x_continuous(breaks = seq(0, 20, by = 5), limits=c(0,20))+ 
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))

```



```
#####
#-----#
# Plastic exposure group -Flaming

Gdata$PLF <- Gdata$PLF %>% mutate(ProbeID = rownames(Gdata$PLF))

GdataPLf <- Gdata$PLF #Plastic

GdataPLf <- select(GdataPLf, ProbeID, logFC)
rownames(GdataPLf) <- NULL

#making ranked gene list
genelist_GdataPLf = GdataPLf[,2] #numeric vector
names(genelist_GdataPLf) = as.character(GdataPLf[,1]) #named vector
genelist_GdataPLf = sort(genelist_GdataPLf, decreasing = TRUE) #must sort in descending order

#Performing GSEA analysis
#Gene Ontology (GO)
gseGO_PLf_ALL <- gseGO(geneList=genelist_GdataPLf,
                           ont ="ALL",
                           keyType = "ENSEMBL",
                           minGSSize = 10, #min size of gene sets for analysis
                           maxGSSize = 500, #max size of gene sets for analysis
                           pvalueCutoff = 0.05,
                           eps=0,
                           verbose = TRUE,
```

```
OrgDb = organism,
pAdjustMethod = "BH") #Benjamini Hochberg adjustment
```

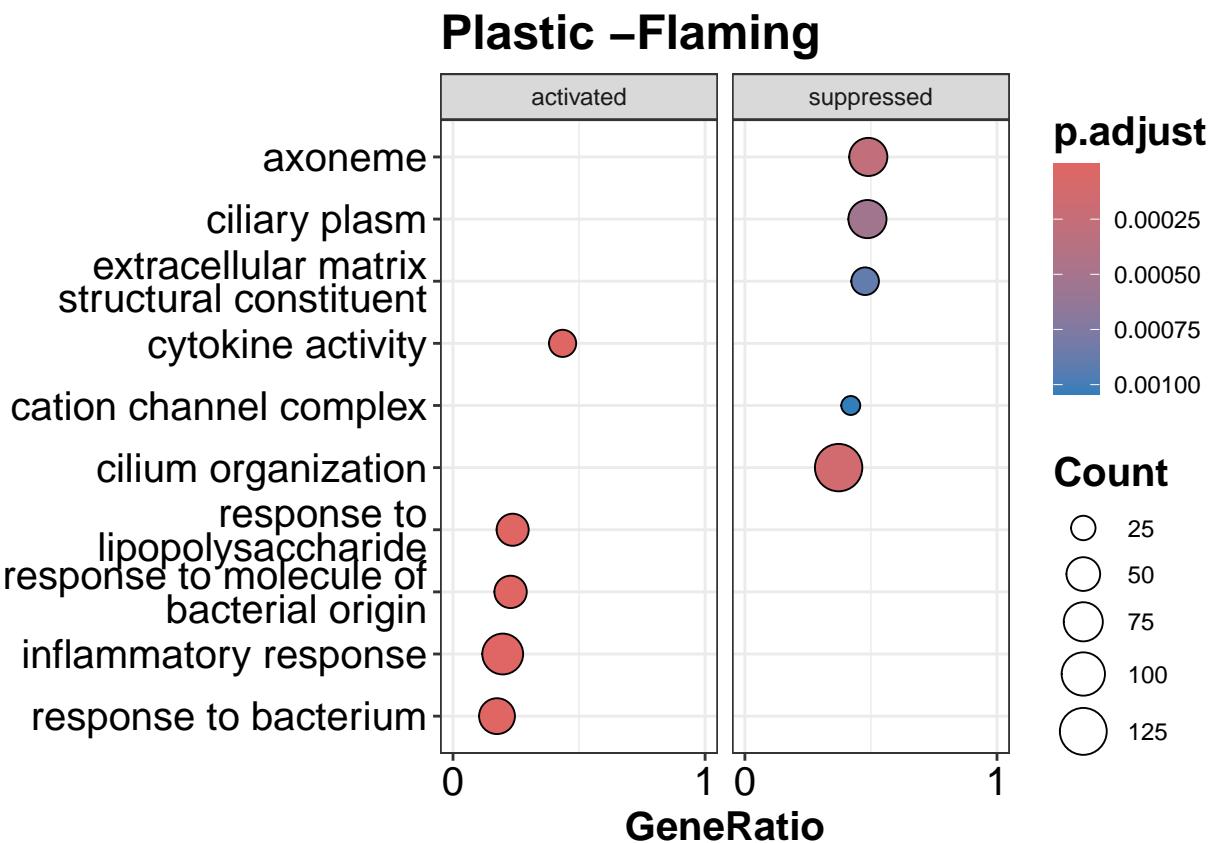
```
## preparing geneSet collections...
```

```
## GSEA analysis...
```

```
## leading edge analysis...
```

```
## done...
```

```
#dotplot
enrichplot::dotplot(gseGO_PLf_ALL, split=".sign", title = "Plastic -Flaming",
                     showCategory = 5, font.size = 15,
                     label_format = 25) + facet_grid(.~.sign) +
  scale_x_continuous(breaks = seq(0, 1, by = 1), limits=c(0,1)) +
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))
```



```
#-----#
```

```
#GO over-representation analysis
```

```
genePLf <- names(genelist_GdataPLf[genelist_GdataPLf[] > 0.5]) # logFC > 0.5
```

```

enGOPLf <- enrichGO(gene      = genePLf,
                     OrgDb     = org.Hs.eg.db,
                     keyType   = 'ENSEMBL',
                     ont       = "ALL",
                     pAdjustMethod = "BH",
                     pvalueCutoff  = 0.05,
                     qvalueCutoff   = 0.1)
head(enGOPLf)

##          ONTOLOGY      ID
## GO:0032496    BP GO:0032496
## GO:0002237    BP GO:0002237
## GO:0010575    BP GO:0010575
## GO:0070486    BP GO:0070486
## GO:1990266    BP GO:1990266
## GO:0050900    BP GO:0050900
##                                         Description
## GO:0032496             response to lipopolysaccharide
## GO:0002237             response to molecule of bacterial origin
## GO:0010575 positive regulation of vascular endothelial growth factor production
## GO:0070486                  leukocyte aggregation
## GO:1990266                  neutrophil migration
## GO:0050900                  leukocyte migration
##          GeneRatio    BgRatio      pvalue      p.adjust      qvalue
## GO:0032496    12/58 412/21261 9.538469e-10 1.545232e-06 1.037183e-06
## GO:0002237    12/58 446/21261 2.334584e-09 1.891013e-06 1.269276e-06
## GO:0010575    5/58  31/21261 2.038326e-08 1.100696e-05 7.388038e-06
## GO:0070486    4/58  14/21261 4.889250e-08 1.980146e-05 1.329104e-05
## GO:1990266    7/58  148/21261 1.534445e-07 4.971603e-05 3.337015e-05
## GO:0050900    10/58 463/21261 4.458665e-07 1.152430e-04 7.735281e-05
##
## GO:0032496 ENSG00000140465/ENSG00000136244/ENSG00000108342/ENSG00000143546/ENSG00000125538/ENSG00000
## GO:0002237 ENSG00000140465/ENSG00000136244/ENSG00000108342/ENSG00000143546/ENSG00000125538/ENSG00000
## GO:0010575
## GO:0070486
## GO:1990266
## GO:0050900
##          Count
## GO:0032496    12
## GO:0002237    12
## GO:0010575     5
## GO:0070486     4
## GO:1990266     7
## GO:0050900    10

#barplot
barplot(enGOPLf, showCategory=10, size = 15, title = "Plastic -flaming")+
  scale_x_continuous(breaks = seq(0, 20, by = 5), limits=c(0,20))+
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))

```

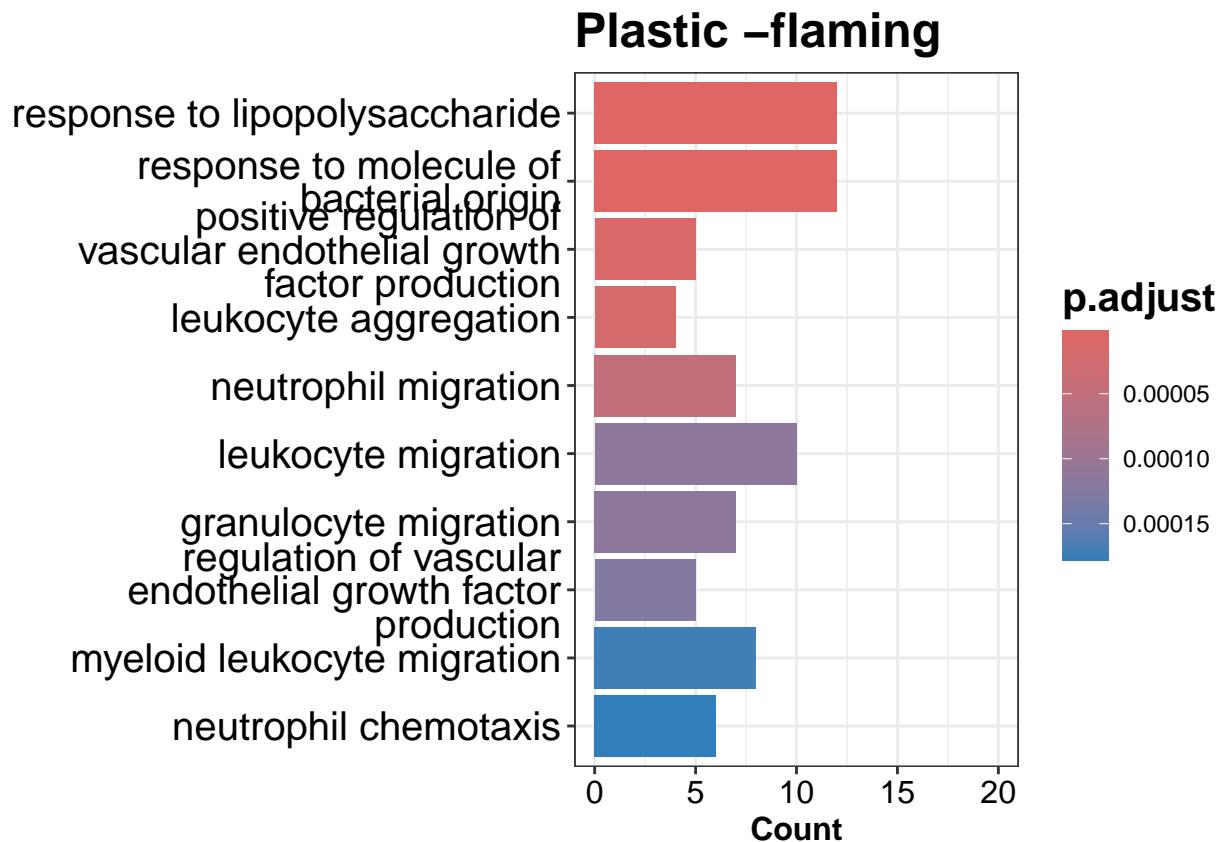
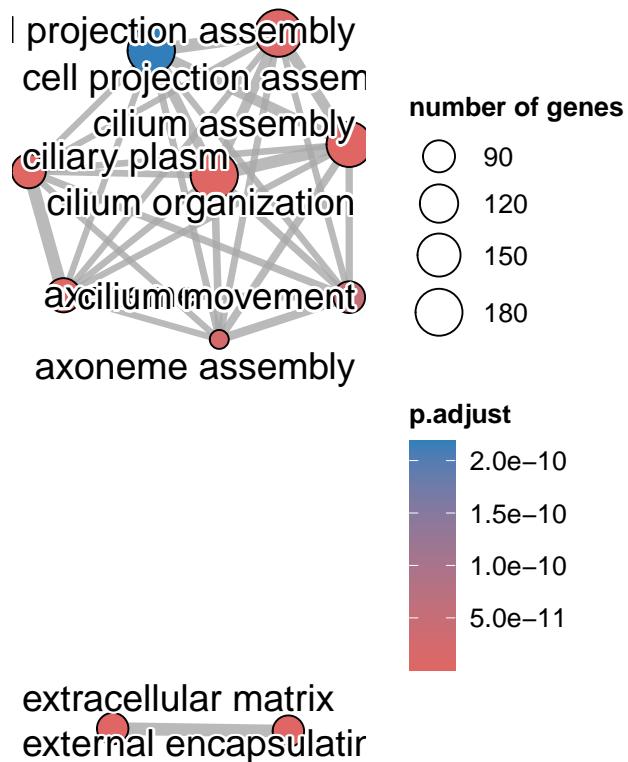


FIGURE E2

```
#-----#
# Enrichment Map:

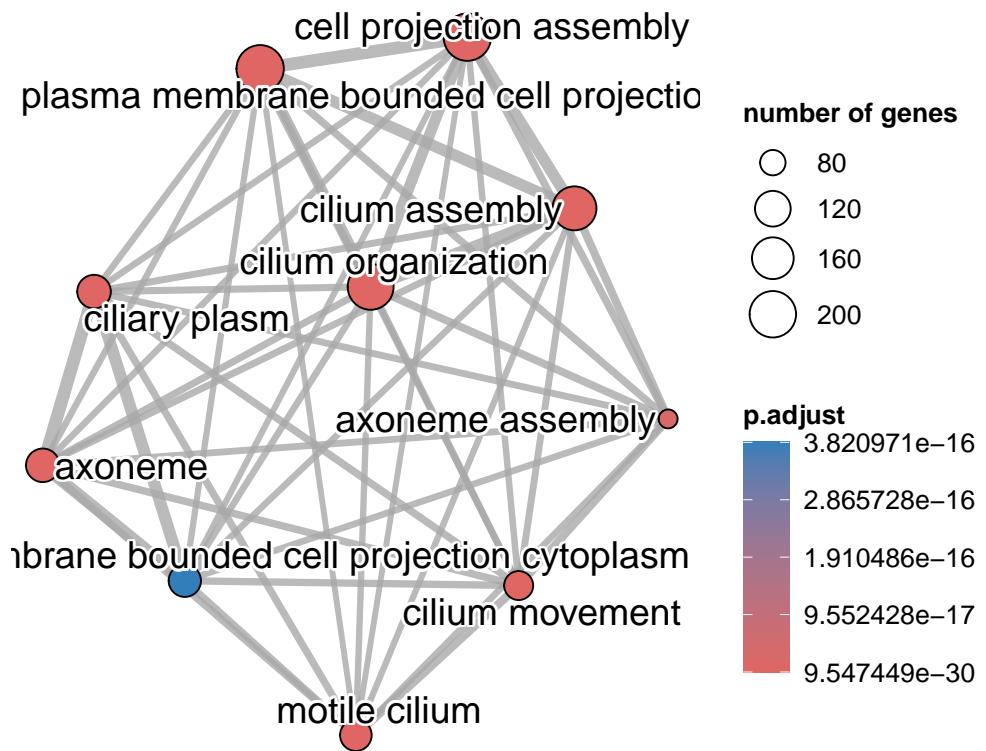
gse1 <- pairwise_termsim(gseGO_CBF_ALL)
emapplot(gse1, showCategory = 10) + ggtitle("Cardboard -flaming")+
  theme(title = element_text(size = 15, face="bold"))
```

Cardboard –flaming



```
gse2 <- pairwise_termsim(gseGO_PWf_ALL)
emapplot(gse2, showCategory = 10) + ggtitle("Plywood -flaming")+
  theme(title = element_text(size = 15, face="bold"))
```

Plywood -flaming



```
gse3 <- pairwise_termsim(gseGO_PLf_ALL)
emappplot(gse3, showCategory = 10) + ggtitle("Plastic -flaming")+
  theme(title = element_text(size = 15, face="bold"))
```

Plastic –flaming

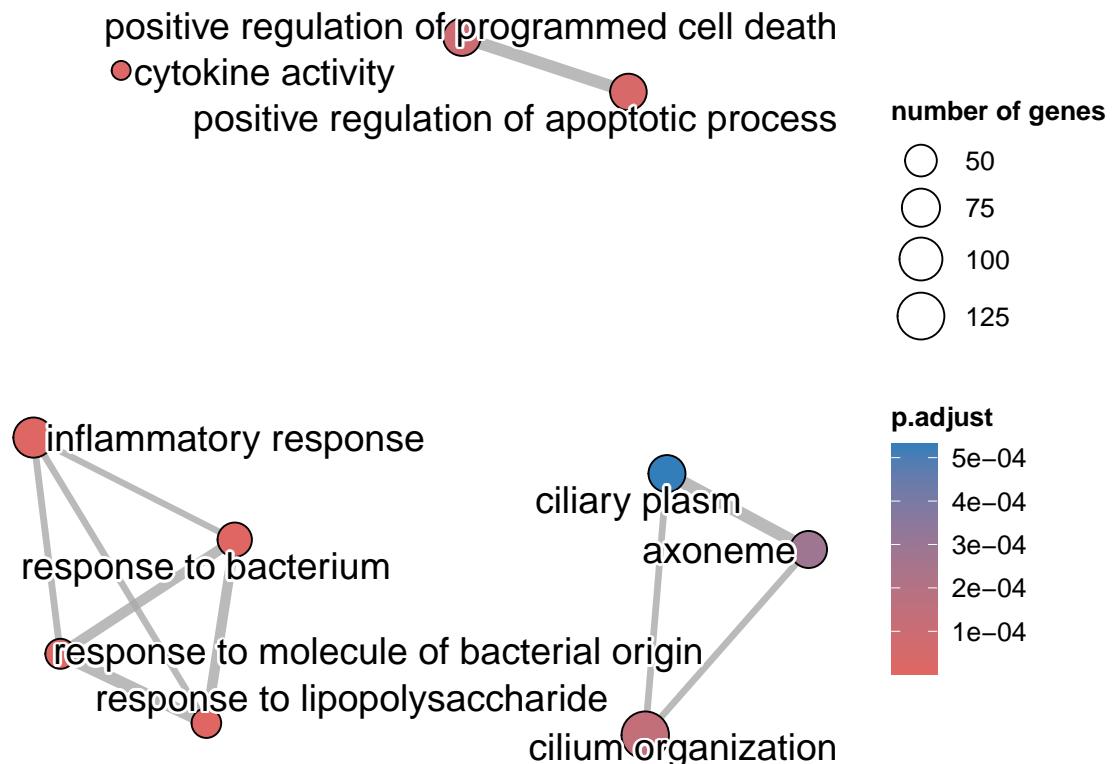


FIGURE E3

```
a4S <- a4 %>%
  keep(str_detect(names(a4), "Smoldering"))
names(a4S) <- sub('\nSmoldering','', names(a4S))

EP_All_S <- euler(a4S, shape = "ellipse") # Euler plot -smoldering
plot(EP_All_S, quantities = TRUE, cex = 5, labels = list(font = 3, cex = 1.5))
```

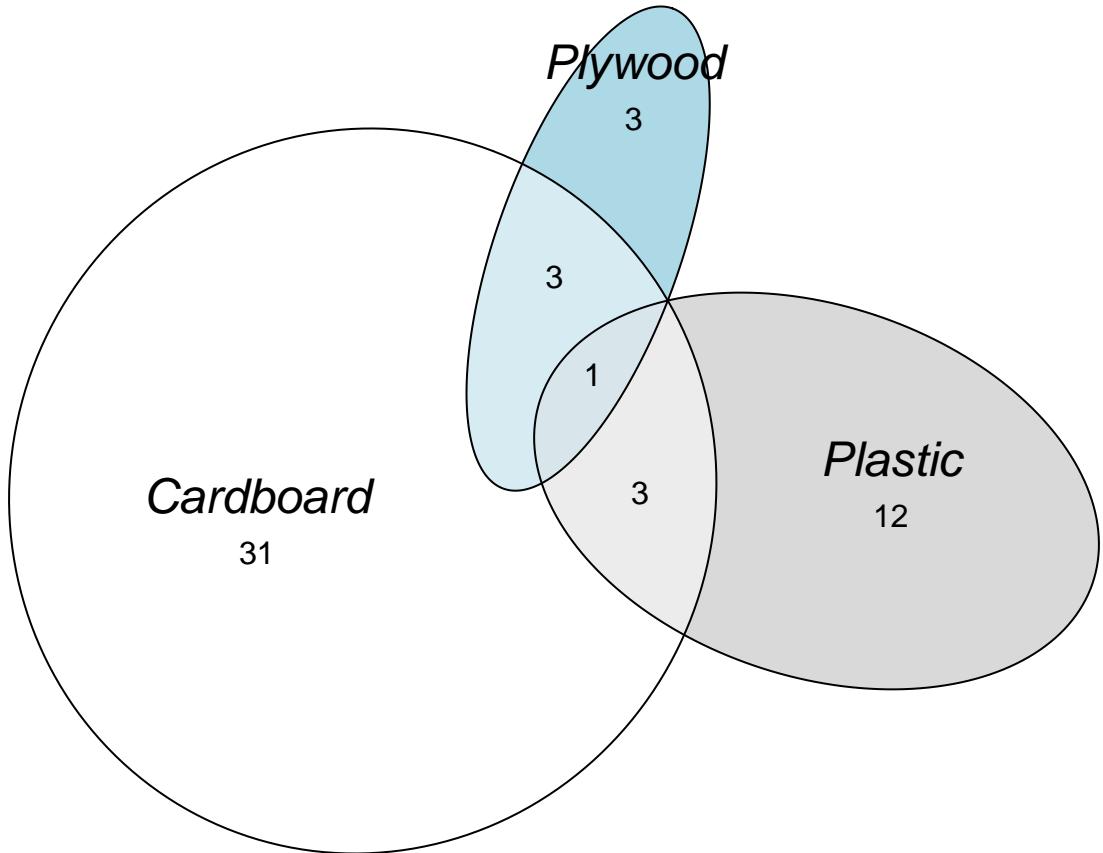


FIGURE E4

```

#-----#
# Smoldering #
#-----#

# Cardboard exposure group -Smoldering
Gdata$CBS <- Gdata$CBS %>% mutate(ProbeID = rownames(Gdata$CBS))
GdataCBs <- Gdata$CBS #Cardboard
GdataCBs <- select(GdataCBs, ProbeID, logFC)
rownames(GdataCBs) <- NULL

#making ranked gene list
genelist_GdataCBs = GdataCBs[,2] #numeric vector
names(genelist_GdataCBs) = as.character(GdataCBs[,1]) #named vector
genelist_GdataCBs = sort(genelist_GdataCBs, decreasing = TRUE) #must sort in descending order

#Performing GSEA analysis
#Gene Ontology (GO)
gseGO_CBS_ALL <- gseGO(geneList=genelist_GdataCBs,
                           ont ="ALL",
                           keyType = "ENSEMBL",
                           minGSSize = 10, #min size of gene sets for analysis
                           maxGSSize = 500, #max size of gene sets for analysis
                           pvalueCutoff = 0.05,
                           eps = 0,
                           )

```

```

    verbose = TRUE,
    OrgDb = organism,
    pAdjustMethod = "BH") #Benjamini Hochberg adjustment

## preparing geneSet collections...

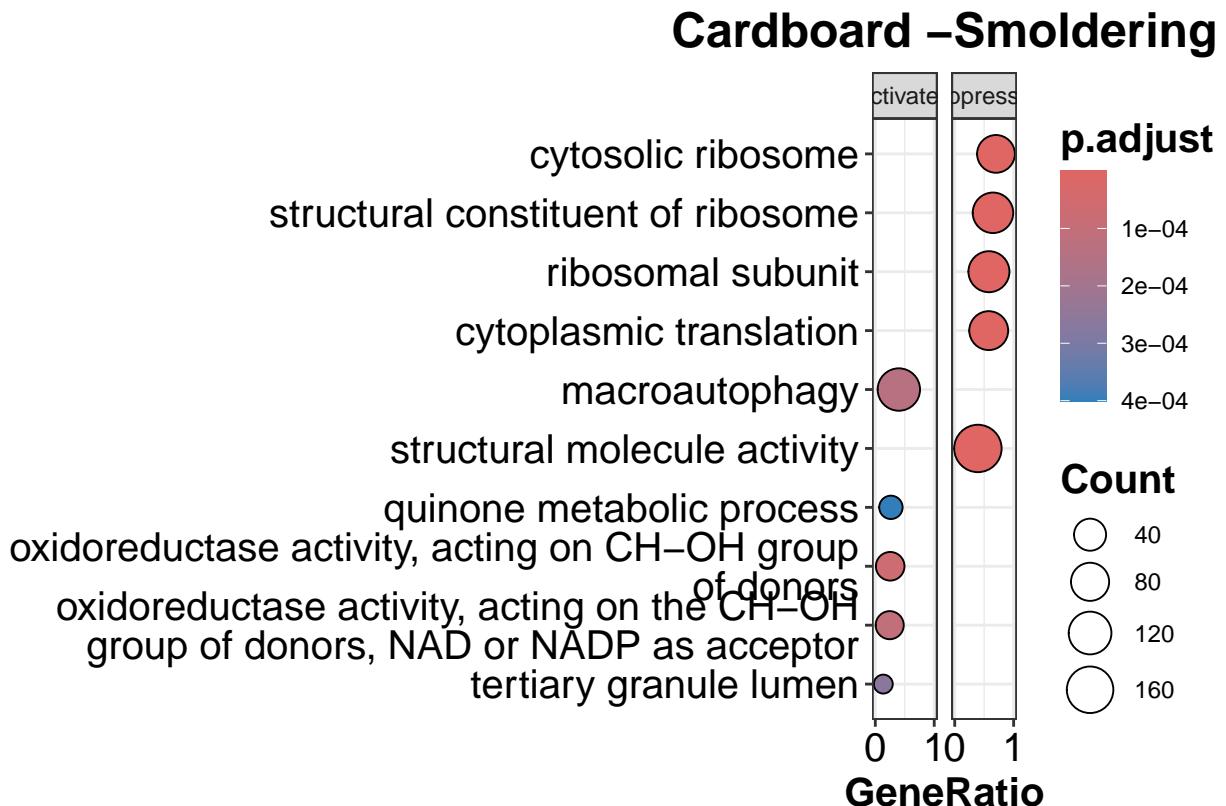
## GSEA analysis...

## leading edge analysis...

## done...

#dotplot
enrichplot::dotplot(gseGO_CBs_ALL, split=".sign", title = "Cardboard -Smoldering",
                     showCategory = 5, font.size = 15, label_format = 50) + facet_grid(.~.sign) +
  scale_x_continuous(breaks = seq(0, 1, by = 1), limits=c(0,1))+
  theme(axis.text.y = element_text(lineheight = 0.80, size = 15),
        title = element_text(size = 15, face="bold"),
        plot.title = element_text(hjust=0.5))+ 
  coord_fixed(ratio = 1, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on")

```



```

#GO over-representation analysis
geneCBs <- names(genelist_GdataCBs[genelist_GdataCBs[] > 0.5]) # logFC > 0.5

```

```

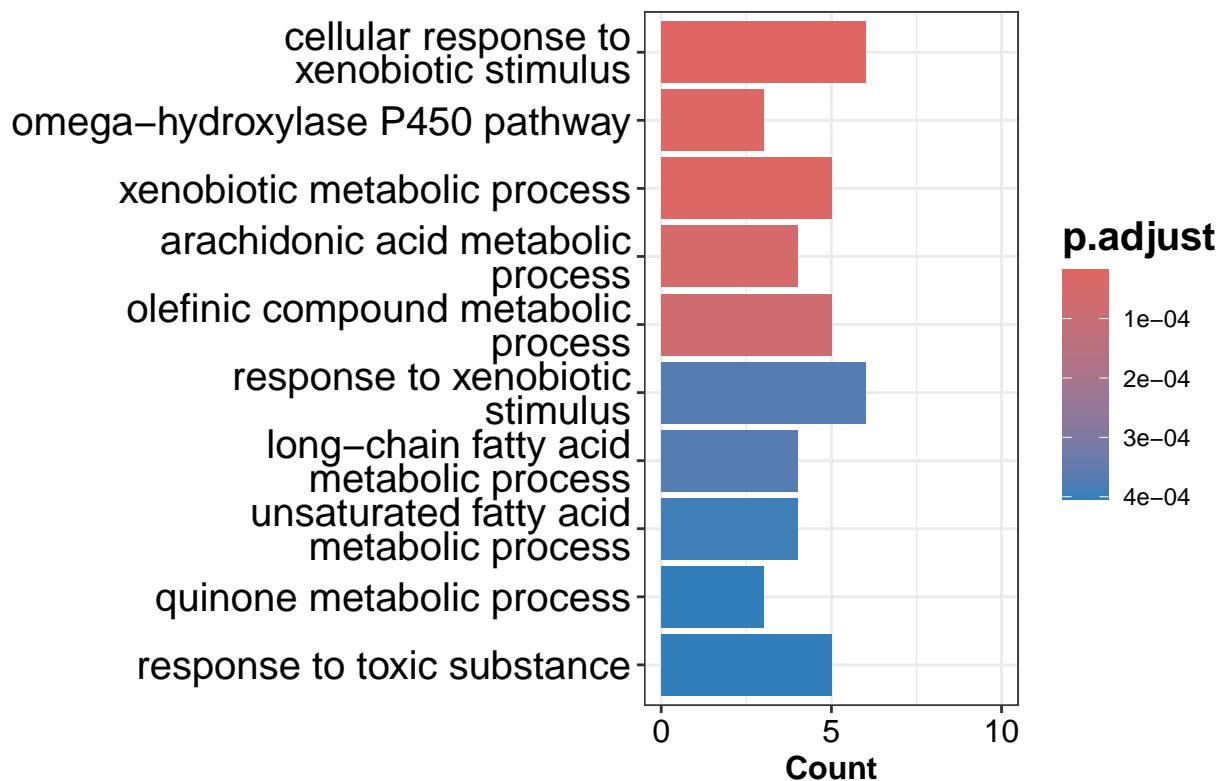
enGOCBs <- enrichGO(gene      = geneCBs,
                     OrgDb     = org.Hs.eg.db,
                     keyType   = 'ENSEMBL',
                     ont       = "ALL",
                     pAdjustMethod = "BH",
                     pvalueCutoff  = 0.05,
                     qvalueCutoff   = 0.1)
head(enGOCBs)

##          ONTOLOGY      ID           Description
## GO:0071466    BP GO:0071466 cellular response to xenobiotic stimulus
## GO:0097267    BP GO:0097267      omega-hydroxylase P450 pathway
## GO:0006805    BP GO:0006805      xenobiotic metabolic process
## GO:0019369    BP GO:0019369      arachidonic acid metabolic process
## GO:0120254    BP GO:0120254      olefinic compound metabolic process
## GO:0009410    BP GO:0009410      response to xenobiotic stimulus
##          GeneRatio    BgRatio      pvalue    p.adjust      qvalue
## GO:0071466    6/20 208/21261 2.820202e-08 1.785188e-05 1.151830e-05
## GO:0097267    3/20 10/21261 8.505967e-08 2.692138e-05 1.737008e-05
## GO:0006805    5/20 133/21261 1.277058e-07 2.694593e-05 1.738592e-05
## GO:0019369    4/20 64/21261 3.488363e-07 5.520335e-05 3.561802e-05
## GO:0120254    5/20 183/21261 6.244366e-07 7.905368e-05 5.100661e-05
## GO:0009410    6/20 476/21261 3.622646e-06 3.687790e-04 2.379417e-04
##
## GO:0071466 ENSG00000154277/ENSG00000140465/ENSG00000138061/ENSG00000108846/ENSG00000108602/ENSG00000
## GO:0097267                                         ENSG00000140465/ENSG00000138061/ENSG00000
## GO:0006805                                         ENSG00000140465/ENSG00000138061/ENSG00000108846/ENSG00000108602/ENSG00000
## GO:0019369                                         ENSG00000140465/ENSG00000138061/ENSG00000186529/ENSG00000
## GO:0120254                                         ENSG00000198074/ENSG00000140465/ENSG00000138061/ENSG00000186529/ENSG00000
## GO:0009410 ENSG00000154277/ENSG00000140465/ENSG00000138061/ENSG00000108846/ENSG00000108602/ENSG00000
##          Count
## GO:0071466    6
## GO:0097267    3
## GO:0006805    5
## GO:0019369    4
## GO:0120254    5
## GO:0009410    6

#barplot
barplot(enGOCBs, showCategory=10, size = 15, title = "Cardboard -Smoldering")+
  scale_x_continuous(breaks = seq(0, 10, by = 5), limits=c(0,10))+
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))+ coord_fixed(2)

```

Cardboard –Smoldering



```

#-----#
# Plywood exposure group -Smoldering

Gdata$PWS <- Gdata$PWS %>% mutate(ProbeID = rownames(Gdata$PWS))
GdataPWS <- Gdata$PWS #Plywood
GdataPWS <- select(GdataPWS, ProbeID, logFC)
rownames(GdataPWS) <- NULL

#making ranked gene list
genelist_GdataPWS = GdataPWS[,2] #numeric vector
names(genelist_GdataPWS) = as.character(GdataPWS[,1]) #named vector
genelist_GdataPWS = sort(genelist_GdataPWS, decreasing = TRUE) #must sort in descending order

#Performing GSEA analysis
#Gene Ontology (GO)
gseGO_PWs_ALL <- gseGO(geneList=genelist_GdataPWS,
                           ont ="ALL",
                           keyType = "ENSEMBL",
                           minGSSize = 10, #min size of gene sets for analysis
                           maxGSSize = 500, #max size of gene sets for analysis
                           pvalueCutoff = 0.05,
                           eps = 0,
                           verbose = TRUE,
                           OrgDb = organism,
                           pAdjustMethod = "BH") #Benjamini Hochberg adjustment

```

```

## preparing geneSet collections...

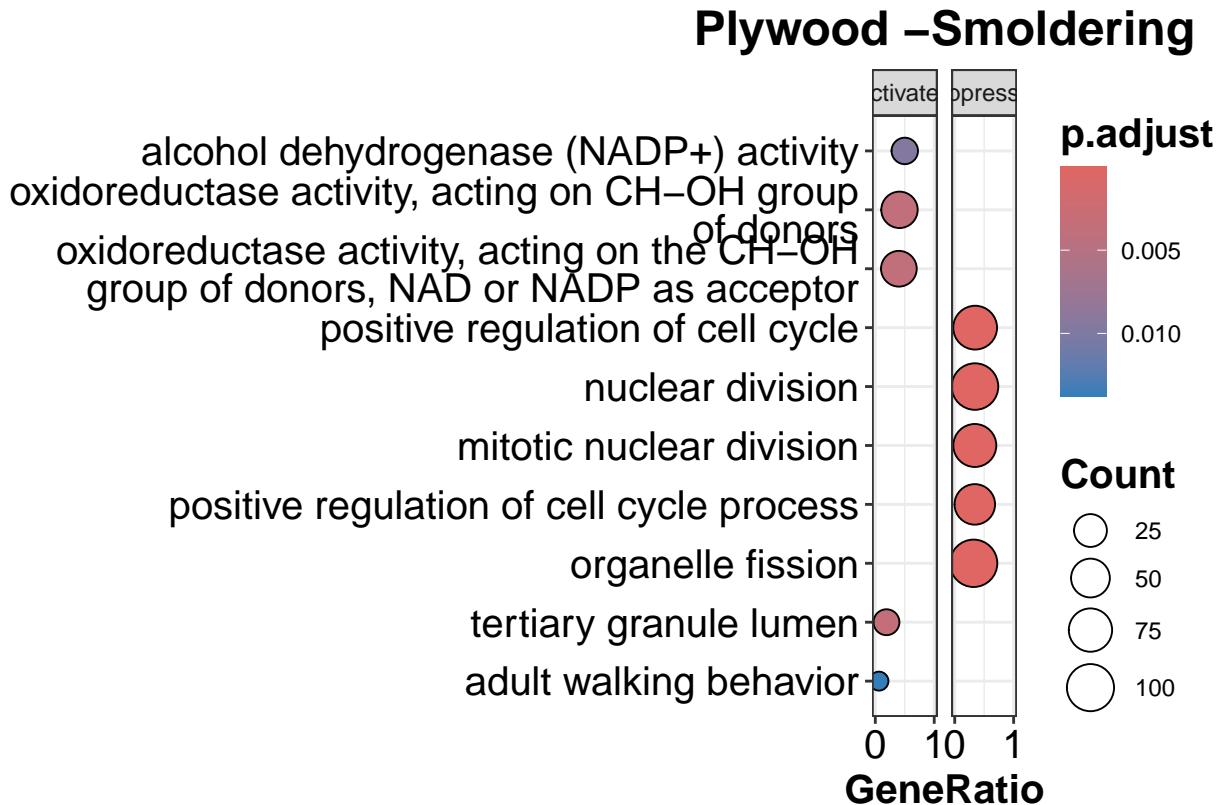
## GSEA analysis...

## leading edge analysis...

## done...

#dotplot
enrichplot::dotplot(gseGO_PWs_ALL, split=".sign", title = "Plywood -Smoldering",
                     showCategory = 5, font.size = 15, label_format = 50) + facet_grid(.~.sign) +
  scale_x_continuous(breaks = seq(0, 1, by = 1), limits=c(0,1))+
  theme(axis.text.y = element_text(lineheight = 0.80, size = 15),
        title = element_text(size = 15, face="bold"),
        plot.title = element_text(hjust=0.5))+
  coord_fixed(ratio = 1, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on")

```



```

#GO over-representation analysis
genePWs <- names(genelist_GdataPWs[genelist_GdataPWs[] > 0.5]) # logFC > 0.5
enGOPWs <- enrichGO(gene      = genePWs,
                      OrgDb     = org.Hs.eg.db,
                      keyType   = 'ENSEMBL',
                      ont       = "ALL",
                      pAdjustMethod = "BH",

```

```

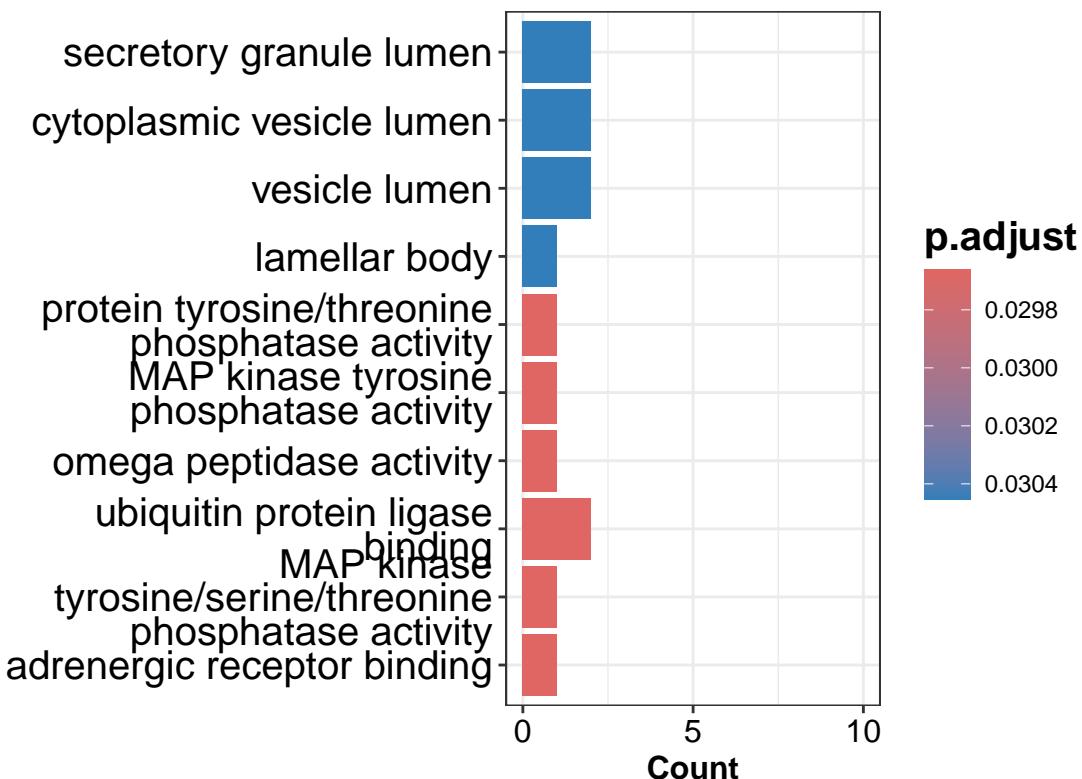
    pvalueCutoff = 0.05,
    qvalueCutoff = 0.1)
head(enGOPWs)

##          ONTOLOGY      ID                  Description
## GO:0034774    CC GO:0034774      secretory granule lumen
## GO:0060205    CC GO:0060205      cytoplasmic vesicle lumen
## GO:0031983    CC GO:0031983      vesicle lumen
## GO:0042599    CC GO:0042599      lamellar body
## GO:0008330    MF GO:0008330 protein tyrosine/threonine phosphatase activity
## GO:0033550    MF GO:0033550      MAP kinase tyrosine phosphatase activity
##          GeneRatio   BgRatio      pvalue   p.adjust     qvalue
## GO:0034774    2/6 381/22568 0.004076742 0.03045377 0.011872815
## GO:0060205    2/6 384/22568 0.004139803 0.03045377 0.011872815
## GO:0031983    2/6 385/22568 0.004160925 0.03045377 0.011872815
## GO:0042599    1/6 17/22568 0.004511670 0.03045377 0.011872815
## GO:0008330    1/7 14/21059 0.004644982 0.02966200 0.009757238
## GO:0033550    1/7 14/21059 0.004644982 0.02966200 0.009757238
##          geneID Count
## GO:0034774 ENSG00000102837/ENSG00000173110      2
## GO:0060205 ENSG00000102837/ENSG00000173110      2
## GO:0031983 ENSG00000102837/ENSG00000173110      2
## GO:0042599           ENSG00000168878      1
## GO:0008330           ENSG00000158050      1
## GO:0033550           ENSG00000158050      1

#barplot
barplot(enGOPWs, showCategory=10, size = 15, title = "Plywood -Smoldering")+
  scale_x_continuous(breaks = seq(0, 10, by = 5), limits=c(0,10))+
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))+ coord_fixed(2)

```

Plywood –Smoldering



```

#--#
# Plastic exposure group -Smoldering

Gdata$PLS <- Gdata$PLS %>% mutate(ProbeID = rownames(Gdata$PLS))
GdataPLs <- Gdata$PLS #Plywood
GdataPLs <- select(GdataPLs, ProbeID, logFC)
rownames(GdataPLs) <- NULL

#making ranked gene list
genelist_GdataPLs = GdataPLs[,2] #numeric vector
names(genelist_GdataPLs) = as.character(GdataPLs[,1]) #named vector
genelist_GdataPLs = sort(genelist_GdataPLs, decreasing = TRUE) #must sort in descending order

#Performing GSEA analysis
#Gene Ontology (GO)
gseGO_PLs_ALL <- gseGO(geneList=genelist_GdataPLs,
                           ont = "ALL",
                           keyType = "ENSEMBL",
                           minGSSize = 10, #min size of gene sets for analysis
                           maxGSSize = 500, #max size of gene sets for analysis
                           pvalueCutoff = 0.05,
                           eps = 0,
                           verbose = TRUE,
                           OrgDb = organism,
                           pAdjustMethod = "BH") #Benjamini Hochberg adjustment

```

```

## preparing geneSet collections...

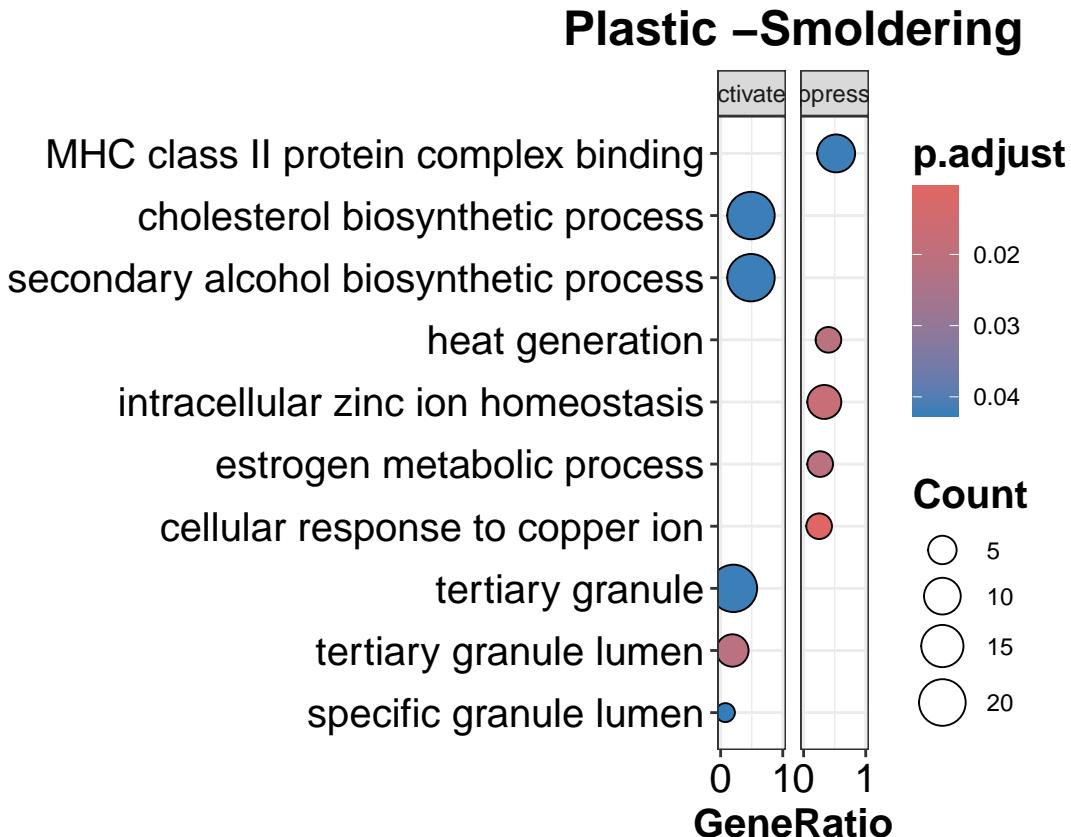
## GSEA analysis...

## leading edge analysis...

## done...

#dotplot
enrichplot::dotplot(gseGO_PLs_ALL, split=".sign", title = "Plastic -Smoldering",
                     showCategory = 5, font.size = 15, label_format = 50) + facet_grid(.~.sign) +
  scale_x_continuous(breaks = seq(0, 1, by = 1), limits=c(0,1)) +
  theme(axis.text.y = element_text(lineheight = 0.80, size = 15),
        title = element_text(size = 15, face="bold"),
        plot.title = element_text(hjust=0.5)) +
  coord_fixed(ratio = 1, xlim = NULL, ylim = NULL, expand = TRUE, clip = "on")

```



```

#GO over-representation analysis
genePLs <- names(genelist_GdataPLs[genelist_GdataPLs[] > 0.5]) # logFC > 0.5
enGOPLs <- enrichGO(gene      = genePLs,
                     OrgDb     = org.Hs.eg.db,
                     keyType   = 'ENSEMBL',
                     ont       = "ALL",
                     pAdjustMethod = "BH",

```

```

        pvalueCutoff = 0.05,
        qvalueCutoff = 0.1)
head(enGOPLs)

##          ONTOLOGY      ID      Description GeneRatio   BgRatio
## GO:0045171      CC GO:0045171 intercellular bridge      2/7 104/22568
##                  pvalue    p.adjust     qvalue                      geneID
## GO:0045171 0.000435085 0.00783153 0.005037827 ENSG00000102837/ENSG00000167755
##                  Count
## GO:0045171      2

#barplot
barplot(enGOPLs, showCategory=10, font = 15, title = "Plastic -Smoldering")+
  scale_x_continuous(breaks = seq(0, 10, by = 5), limits=c(0,10))+
  theme(axis.text.y = element_text(lineheight = 0.7, size = 15),
        title = element_text(size = 15, face="bold"))+ coord_fixed(2)

```

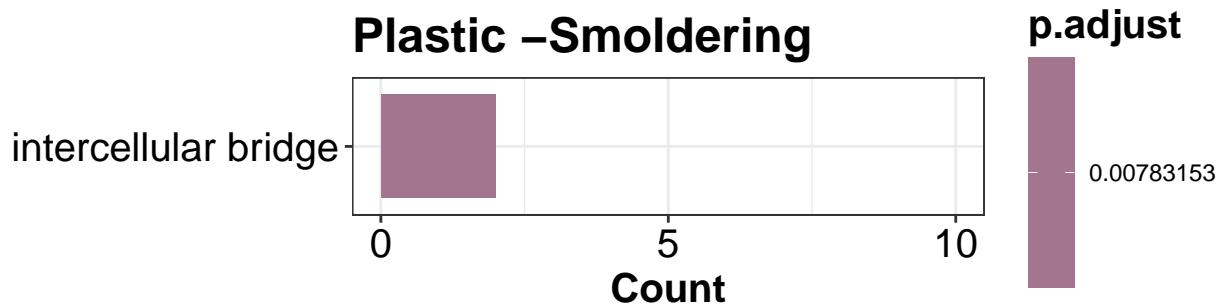


Figure 4

```

#####
# CORRELATION WITH CHEMICAL COMPOUNDS
#####

# Reading in PAH values
# in burn pit smoke condensates

```

```

ChCs <- read_excel("chemical_concentrations.xlsx",
                     sheet=1,
                     col_names = TRUE)

## New names:
## * `` -> '...1'

ChCs <- as.data.frame(ChCs)
ChCs <- as.matrix(ChCs)
head(ChCs)

##      ...1          Cardboard Flaming Cardboard Smoldering Plastic Flaming
## [1,] "Naphthalene"    "18400"        " 59"           "139425"
## [2,] "Acenaphthylene" " 7000"        " 59"           " 83125"
## [3,] "Acenaphthene"   " 650"         " 59"           " 4450"
## [4,] "Fluorene"       " 3450"        "100"          " 47775"
## [5,] "Phenanthrene"   "38100"        "225"          "195425"
## [6,] "Anthracene"     " 4150"        " 59"           " 36675"
##          Plastic Smoldering Plywood Flaming Plywood Smoldering
## [1,] "1075"           "18150"        " 59"
## [2,] " 59"             " 6225"        " 59"
## [3,] " 200"            " 400"         " 59"
## [4,] " 700"            " 2625"        "125"
## [5,] "1075"           "32425"        "150"
## [6,] " 59"             " 3100"        " 59"

row.names(ChCs) <- ChCs[,1]

ChCs <- ChCs[,2:ncol(ChCs)]
ChCs <- t(ChCs)

head(ChCs)

##          Naphthalene Acenaphthylene Acenaphthene Fluorene
## Cardboard Flaming    "18400"      " 7000"        " 650"      " 3450"
## Cardboard Smoldering " 59"        " 59"         " 59"       "100"
## Plastic Flaming      "139425"     " 83125"       " 4450"     " 47775"
## Plastic Smoldering   "1075"       " 59"         " 200"       " 700"
## Plywood Flaming       "18150"      " 6225"       " 400"       " 2625"
## Plywood Smoldering   " 59"        " 59"         " 59"       "125"
##          Phenanthrene Anthracene Fluoranthene Pyrene
## Cardboard Flaming     "38100"      " 4150"       "29850"     "26250"
## Cardboard Smoldering " 225"       " 59"        " 59"       " 59"
## Plastic Flaming       "195425"     " 36675"      "77750"     "62775"
## Plastic Smoldering   "1075"       " 59"        " 150"      " 59"
## Plywood Flaming       "32425"      " 3100"       "32975"     "23050"
## Plywood Smoldering   "150"        " 59"        " 59"       " 59"
##          Benz(a)anthracene Chrysene Benzo(b)fluoranthene
## Cardboard Flaming     " 8450"       " 7175"      "10400"
## Cardboard Smoldering " 59"        " 59"        " 59"
## Plastic Flaming       "21825"      " 20200"     "33750"

```

```

## Plastic Smoldering " 59" " 150" " 75"
## Plywood Flaming " 7125" " 7325" "12150"
## Plywood Smoldering " 59" " 59" " 59"
##
## Benzo(k)fluoranthene Benzo(a)pyrene Indeno[1,2,3-cd]pyrene
## Cardboard Flaming " 3250" " 4775" " 3425"
## Cardboard Smoldering " 59" " 59" " 59"
## Plastic Flaming " 7100" " 18600" " 11700"
## Plastic Smoldering " 59" " 59" " 59"
## Plywood Flaming " 3575" " 4575" " 3750"
## Plywood Smoldering " 59" " 59" " 59"
##
## Dibenzo[ah]anthracene Benzo[ghi]perylene
## Cardboard Flaming " 775" " 3800"
## Cardboard Smoldering " 59" " 59"
## Plastic Flaming " 3550" " 14775"
## Plastic Smoldering " 59" " 59"
## Plywood Flaming " 800" " 3475"
## Plywood Smoldering " 59" " 59"
##
## 1,4-Naphthoquinone 1-Naphthalenecarboxaldehyde
## Cardboard Flaming " 1800" " 7650"
## Cardboard Smoldering "225" "100"
## Plastic Flaming " 8525" " 16925"
## Plastic Smoldering " 250" " 200"
## Plywood Flaming " 875" " 6150"
## Plywood Smoldering "150" " 59"
##
## 9-Fluorenone 9,10-Anthraquinone 1,8-Naphthalic anhydride
## Cardboard Flaming " 7825" " 4825" " 7375"
## Cardboard Smoldering "525" "125" " 59"
## Plastic Flaming " 29325" " 6525" " 14325"
## Plastic Smoldering "3925" " 175" " 59"
## Plywood Flaming "13175" " 6975" "17125"
## Plywood Smoldering "250" " 59" " 59"
##
## Benzanthrone 1-Pyrenecarboxaldehyde
## Cardboard Flaming "12950" " 725"
## Cardboard Smoldering " 59" " 59"
## Plastic Flaming " 32025" " 1250"
## Plastic Smoldering " 100" " 59"
## Plywood Flaming "19550" " 975"
## Plywood Smoldering " 59" " 59"
##
## Benz[a]anthracene-7,12-quinone 4-Nitrobiphenyl
## Cardboard Flaming " 500" " 1000"
## Cardboard Smoldering " 59" " 59"
## Plastic Flaming " 75" " 750"
## Plastic Smoldering " 59" " 59"
## Plywood Flaming " 950" " 175"
## Plywood Smoldering " 59" " 59"

```

```
sapply(ChCs, class) #to make sure that ChC columns are numeric
```

```

##      18400      59     139425      1075     18150      59
## "character" "character" "character" "character" "character" "character"
##      7000      59     83125       59      6225      59
## "character" "character" "character" "character" "character" "character"
##      650       59      4450       200      400       59
## "character" "character" "character" "character" "character" "character"

```

```

##      3450       100     47775       700      2625       125
## "character" "character" "character" "character" "character" "character"
##    38100       225     195425      1075      32425       150
## "character" "character" "character" "character" "character" "character"
##    4150        59     36675       59      3100        59
## "character" "character" "character" "character" "character" "character"
##    29850       59     77750       150      32975       59
## "character" "character" "character" "character" "character" "character"
##    26250       59     62775       59      23050       59
## "character" "character" "character" "character" "character" "character"
##    8450        59     21825       59      7125        59
## "character" "character" "character" "character" "character" "character"
##    7175        59     20200       150      7325        59
## "character" "character" "character" "character" "character" "character"
##   10400       59     33750       75      12150       59
## "character" "character" "character" "character" "character" "character"
##    3250        59     7100        59      3575        59
## "character" "character" "character" "character" "character" "character"
##    4775        59     18600       59      4575        59
## "character" "character" "character" "character" "character" "character"
##    3425        59     11700       59      3750        59
## "character" "character" "character" "character" "character" "character"
##    775         59     3550        59      800         59
## "character" "character" "character" "character" "character" "character"
##    3800        59     14775       59      3475        59
## "character" "character" "character" "character" "character" "character"
##    1800       225     8525        250      875        150
## "character" "character" "character" "character" "character" "character"
##    7650       100     16925       200      6150        59
## "character" "character" "character" "character" "character" "character"
##    7825       525     29325      3925      13175       250
## "character" "character" "character" "character" "character" "character"
##    4825       125     6525        175      6975        59
## "character" "character" "character" "character" "character" "character"
##    7375        59     14325       59      17125       59
## "character" "character" "character" "character" "character" "character"
##   12950       59     32025       100      19550       59
## "character" "character" "character" "character" "character" "character"
##    725         59     1250        59      975         59
## "character" "character" "character" "character" "character" "character"
##    500         59      75         59      950         59
## "character" "character" "character" "character" "character" "character"
##   1000        59     750         59      175        59
## "character" "character" "character" "character" "character" "character"

mode(ChCs) <- "numeric" # Changing matrix to numeric

#-----#
# Reading in inorganic element values
# in burn pit smoke condensates

IEs <- read_excel("chemical_concentrations.xlsx",
                  sheet=2,
                  col_names = TRUE)

```

```

## New names:
## * `` -> '...1'

IEs <- as.data.frame(IEs)
IEs <- as.matrix(IEs)
head(IEs)

##          ...1      Cardboard Flaming Cardboard Smoldering Plastic Flaming
## [1,] "Silver(Ag)"    "4.513900e+01"    "4.079801e+00"    "7.464783e+00"
## [2,] "Aluminium(Al)" "1.895869e+05"    "1.055825e+05"    "1.657821e+05"
## [3,] "Barium(Ba)"    "3.647548e+03"    "5.598425e+02"    "3.971390e+02"
## [4,] "Bismuth(Bi)"   "9.224876e+00"    "4.635075e+00"    "4.640270e+00"
## [5,] "Calcium(Ca)"   "5.062324e+05"    "6.842679e+04"    "1.677721e+04"
## [6,] "Cadmium(Cd)"   "1.123106e+03"    "6.247276e+00"    "3.244178e+01"
##          Plastic Smoldering Plywood Flaming Plywood Smoldering
## [1,]      5.456312"    "9.112535e+01"    "6.085101e+00"
## [2,] "30944.772107"  "1.881952e+05"    "1.337137e+05"
## [3,]    196.861092"   "3.100334e+03"    "3.279433e+02"
## [4,]     4.673249"    "4.634007e+00"    "4.618102e+00"
## [5,] "33522.658482"  "4.512605e+05"    "4.276848e+04"
## [6,]     6.298726"    "1.616845e+02"    "6.224398e+00"

row.names(IEs) <- IEs[, 1]

IEs <- IEss[, 2:ncol(IEs)]
IEs <- t(IEs)

head(IEs)

##          Silver(Ag)      Aluminium(Al)      Barium(Ba)
## Cardboard Flaming    "4.513900e+01"    "1.895869e+05"    "3.647548e+03"
## Cardboard Smoldering "4.079801e+00"    "1.055825e+05"    "5.598425e+02"
## Plastic Flaming      "7.464783e+00"    "1.657821e+05"    "3.971390e+02"
## Plastic Smoldering   "5.456312"    "30944.772107"    "196.861092"
## Plywood Flaming       "9.112535e+01"    "1.881952e+05"    "3.100334e+03"
## Plywood Smoldering   "6.085101e+00"    "1.337137e+05"    "3.279433e+02"
##          Bismuth(Bi)      Calcium(Ca)      Cadmium(Cd)
## Cardboard Flaming    "9.224876e+00"    "5.062324e+05"    "1.123106e+03"
## Cardboard Smoldering "4.635075e+00"    "6.842679e+04"    "6.247276e+00"
## Plastic Flaming       "4.640270e+00"    "1.677721e+04"    "3.244178e+01"
## Plastic Smoldering   "4.673249"    "33522.658482"    "6.298726"
## Plywood Flaming       "4.634007e+00"    "4.512605e+05"    "1.616845e+02"
## Plywood Smoldering   "4.618102e+00"    "4.276848e+04"    "6.224398e+00"
##          Cobalt(Co)      Chromium(Cr)      Copper(Cu)
## Cardboard Flaming    "3.691271e+01"    "1.496991e+03"    "2.951415e+04"
## Cardboard Smoldering "5.437271e+01"    "4.960772e+02"    "5.863243e+03"
## Plastic Flaming       "5.073116e+00"    "1.748774e+02"    "9.130588e+03"
## Plastic Smoldering   "15.531724"    "111.679455"    "1568.788335"
## Plywood Flaming       "5.804661e+01"    "1.309426e+03"    "3.842478e+04"
## Plywood Smoldering   "1.823565e+01"    "3.093518e+02"    "1.255228e+04"
##          Iron(Fe)      Potassium(K)      Magnesium(Mg)
## Cardboard Flaming    "1.738679e+04"    "4.805980e+04"    "6.615910e+04"
## Cardboard Smoldering "1.245742e+04"    "6.683411e+03"    "1.588520e+04"

```

```

## Plastic Flaming      "2.209968e+03" "3.201848e+03" "4.519769e+03"
## Plastic Smoldering "2693.602834"  "2311.852056"  "6887.049612"
## Plywood Flaming     "1.529375e+04" "4.826972e+04" "6.489042e+04"
## Plywood Smoldering "6.064904e+03" "4.376809e+03" "1.321981e+04"
##
## Manganese(Mn)   Molybdenum(Mo) Sodium(Na)
## Cardboard Flaming  "5.064113e+02" "3.107438e+01" "8.000476e+05"
## Cardboard Smoldering "2.110814e+02" "2.514149e+01" "1.881778e+05"
## Plastic Flaming    "6.024994e+01" "3.193461e+01" "2.242862e+05"
## Plastic Smoldering "119.498101"  "22.350319"  "55207.111870"
## Plywood Flaming     "1.001207e+03" "3.273762e+01" "1.099099e+06"
## Plywood Smoldering "1.462239e+02" "1.452633e+01" "2.282125e+05"
##
## Nickel(Ni)       Phosphorus(P) Lead(Pb)
## Cardboard Flaming "9.764304e+02" "6.489554e+03" "3.251293e+02"
## Cardboard Smoldering "6.441665e+02" "5.683006e+01" "3.984432e+01"
## Plastic Flaming   "1.032966e+02" "5.689375e+01" "4.196020e+01"
## Plastic Smoldering "90.280263"   "57.298090"  "19.707950"
## Plywood Flaming    "1.504843e+03" "6.530460e+03" "2.144221e+02"
## Plywood Smoldering "3.113730e+02" "5.662194e+01" "4.111933e+01"
##
## Sulphur(S)        Antimony(Sb) Silicon(Si)
## Cardboard Flaming "7.276526e+05" "2.334272e+03" "1.546707e+06"
## Cardboard Smoldering "1.026258e+05" "6.191472e+02" "1.991550e+05"
## Plastic Flaming   "2.017509e+04" "4.114115e+03" "1.395676e+06"
## Plastic Smoldering "29442.760558" "221.761464" "39984.992009"
## Plywood Flaming    "1.551957e+05" "1.323120e+03" "1.889320e+06"
## Plywood Smoldering "2.007870e+04" "6.244928e+02" "5.035145e+05"
##
## Tin(Sn)           Strontium(Sr) Titanium(Ti)
## Cardboard Flaming "5.671628e+01" "3.708256e+03" "6.662458e+02"
## Cardboard Smoldering "1.007625e+00" "5.072270e+02" "4.847315e+01"
## Plastic Flaming   "1.008754e+00" "2.397086e+02" "1.210505e+01"
## Plastic Smoldering "1.015924"   "172.462187"  "654.867313"
## Plywood Flaming    "8.881010e+01" "3.969292e+03" "7.884903e+02"
## Plywood Smoldering "1.003935e+00" "3.362286e+02" "1.204722e+01"
##
## Zinc(Zn)
## Cardboard Flaming  "1.056255e+05"
## Cardboard Smoldering "2.531852e+04"
## Plastic Flaming    "2.293783e+04"
## Plastic Smoldering "14483.826023"
## Plywood Flaming    "8.301055e+04"
## Plywood Smoldering "2.847739e+04"

```

```
sapply(IEs, class) #to make sure that IE columns are numeric
```

```

## 4.513900e+01 4.079801e+00 7.464783e+00      5.456312 9.112535e+01 6.085101e+00
## "character" "character" "character" "character" "character" "character"
## 1.895869e+05 1.055825e+05 1.657821e+05 30944.772107 1.881952e+05 1.337137e+05
## "character" "character" "character" "character" "character" "character"
## 3.647548e+03 5.598425e+02 3.971390e+02      196.861092 3.100334e+03 3.279433e+02
## "character" "character" "character" "character" "character" "character"
## 9.224876e+00 4.635075e+00 4.640270e+00      4.673249 4.634007e+00 4.618102e+00
## "character" "character" "character" "character" "character" "character"
## 5.062324e+05 6.842679e+04 1.677721e+04 33522.658482 4.512605e+05 4.276848e+04
## "character" "character" "character" "character" "character" "character"
## 1.123106e+03 6.247276e+00 3.244178e+01      6.298726 1.616845e+02 6.224398e+00
## "character" "character" "character" "character" "character" "character"

```

```

## 3.691271e+01 5.437271e+01 5.073116e+00      15.531724 5.804661e+01 1.823565e+01
## "character" "character" "character" "character" "character" "character" "character"
## 1.496991e+03 4.960772e+02 1.748774e+02      111.679455 1.309426e+03 3.093518e+02
## "character" "character" "character" "character" "character" "character" "character"
## 2.951415e+04 5.863243e+03 9.130588e+03      1568.788335 3.842478e+04 1.255228e+04
## "character" "character" "character" "character" "character" "character" "character"
## 1.738679e+04 1.245742e+04 2.209968e+03      2693.602834 1.529375e+04 6.064904e+03
## "character" "character" "character" "character" "character" "character" "character"
## 4.805980e+04 6.683411e+03 3.201848e+03      2311.852056 4.826972e+04 4.376809e+03
## "character" "character" "character" "character" "character" "character" "character"
## 6.615910e+04 1.588520e+04 4.519769e+03      6887.049612 6.489042e+04 1.321981e+04
## "character" "character" "character" "character" "character" "character" "character"
## 5.064113e+02 2.110814e+02 6.024994e+01      119.498101 1.001207e+03 1.462239e+02
## "character" "character" "character" "character" "character" "character" "character"
## 3.107438e+01 2.514149e+01 3.193461e+01      22.350319 3.273762e+01 1.452633e+01
## "character" "character" "character" "character" "character" "character" "character"
## 8.000476e+05 1.881778e+05 2.242862e+05      55207.111870 1.099099e+06 2.282125e+05
## "character" "character" "character" "character" "character" "character" "character"
## 9.764304e+02 6.441665e+02 1.032966e+02      90.280263 1.504843e+03 3.113730e+02
## "character" "character" "character" "character" "character" "character" "character"
## 6.489554e+03 5.683006e+01 5.689375e+01      57.298090 6.530460e+03 5.662194e+01
## "character" "character" "character" "character" "character" "character" "character"
## 3.251293e+02 3.984432e+01 4.196020e+01      19.707950 2.144221e+02 4.111933e+01
## "character" "character" "character" "character" "character" "character" "character"
## 7.276526e+05 1.026258e+05 2.017509e+04      29442.760558 1.551957e+05 2.007870e+04
## "character" "character" "character" "character" "character" "character" "character"
## 2.334272e+03 6.191472e+02 4.114115e+03      221.761464 1.323120e+03 6.244928e+02
## "character" "character" "character" "character" "character" "character" "character"
## 1.546707e+06 1.9911550e+05 1.395676e+06      39984.992009 1.889320e+06 5.035145e+05
## "character" "character" "character" "character" "character" "character" "character"
## 5.671628e+01 1.007625e+00 1.008754e+00      1.015924 8.881010e+01 1.003935e+00
## "character" "character" "character" "character" "character" "character" "character"
## 3.708256e+03 5.072270e+02 2.397086e+02      172.462187 3.969292e+03 3.362286e+02
## "character" "character" "character" "character" "character" "character" "character"
## 6.662458e+02 4.847315e+01 1.210505e+01      654.867313 7.884903e+02 1.204722e+01
## "character" "character" "character" "character" "character" "character" "character"
## 1.056255e+05 2.531852e+04 2.293783e+04      14483.826023 8.301055e+04 2.847739e+04
## "character" "character" "character" "character" "character" "character" "character"

mode(IEs) <- "numeric" # Changing matrix to numeric

#-----#
counts_ALL <- counts %>% select(!ends_with("CTRL")) # selecting all samples
# except control

counts_ALL <- subset(counts_ALL, rownames(counts_ALL) %in% c(unlist(z[7,3])))

counts_ALL <- counts_ALL %>% rownames_to_column(var = "transcripts")

counts_ALL <- counts_ALL %>% pivot_longer(cols = -1, names_to = "SampleID", values_to = "count")

```

```

counts_ALL$SampleID <- substr(counts_ALL$SampleID, 4, nchar(counts_ALL$SampleID))

counts_ALL$SampleID <- str_replace_all(counts_ALL$SampleID,
                                         c(CBFh = "Cardboard Flaming",
                                           CBSh = "Cardboard Smoldering",
                                           PLFh = "Plastic Flaming",
                                           PLSh = "Plastic Smoldering",
                                           PWFh = "Plywood Flaming",
                                           PWSh = "Plywood Smoldering"))

colnames(counts_ALL)

## [1] "transcripts" "SampleID"      "count"

counts_ALL <- counts_ALL %>%
  group_by(transcripts, SampleID) %>%
  summarise(across(everything(), sum))

## `summarise()` has grouped output by 'transcripts'. You can override using the
## '.groups' argument.

counts_ALL <- counts_ALL %>% pivot_wider(names_from = "SampleID", values_from = "count") %>%
  column_to_rownames(var = "transcripts")

counts_ALL$gene <- mapIds(org.Hs.eg.db, keys = row.names(counts_ALL),
                           keytype = "ENSEMBL", column = "SYMBOL",
                           multiVals="first") #adding gene names

## 'select()' returned 1:1 mapping between keys and columns

counts_ALL <- subset(counts_ALL, counts_ALL$gene != 'NA')
rownames(counts_ALL) <- NULL
rownames(counts_ALL) <- counts_ALL$gene
counts_ALL <- counts_ALL[,1:(ncol(counts_ALL)-1)]

counts_ALL <- t(counts_ALL)

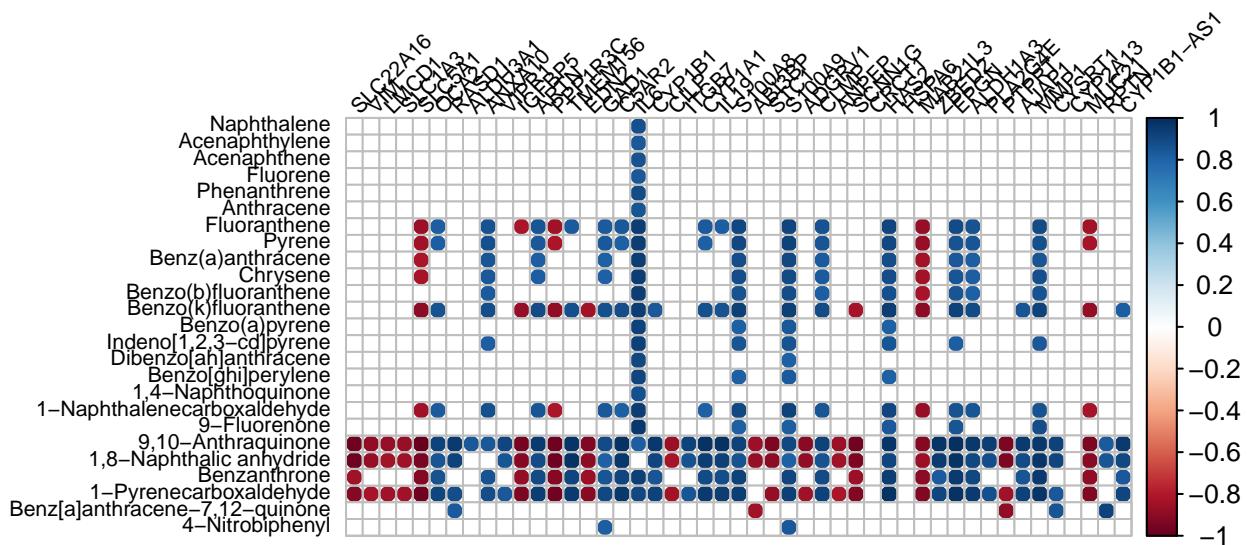
#####
# Pearson' test to detect correlation

cor.matrix <- cor.test(counts_ALL, ChCs,
                        adjust = "none", method = "pearson")

corrplot(t(cor.matrix$r), order="original", tl.col = "black",
          p.mat = t(cor.matrix$p), sig.level = 0.05, insig = "blank",
          title ="PAHs", tl.srt = 45,
          tl.cex = 0.7, mar=c(0,0,1,0)) # significance level p<0.05

```

PAHs



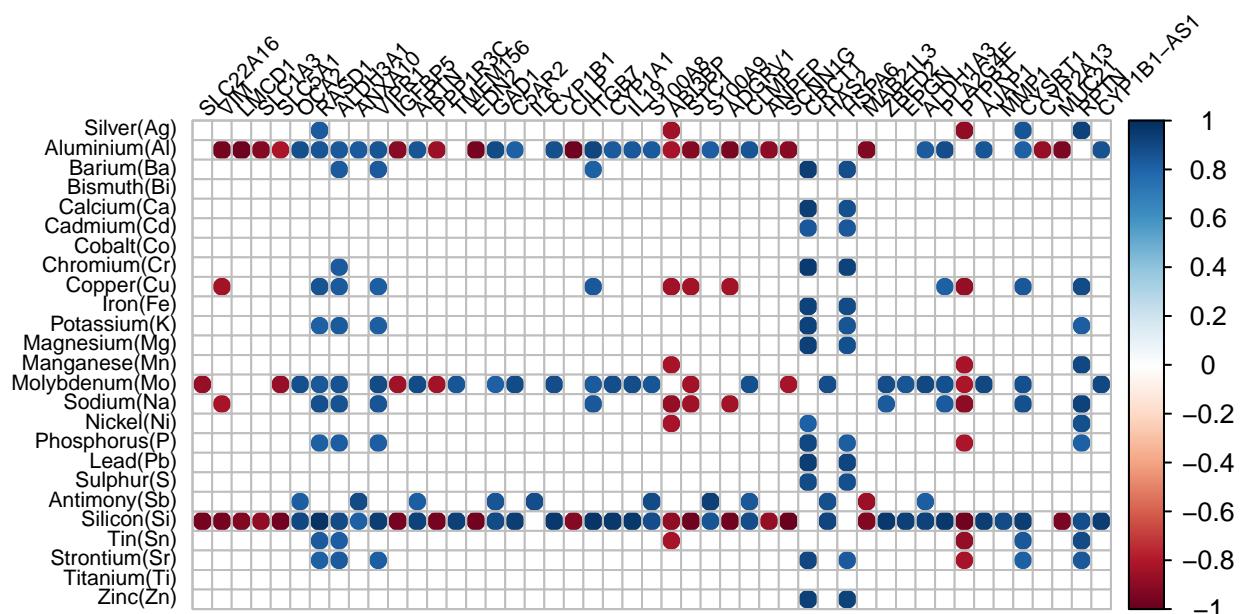
```

cor.matrix2 <- corr.test(counts_ALL, IEs,
                        adjust = "none", method = "pearson")

corrplot(t(cor.matrix2$r), order="original", tl.col = "black",
         p.mat = t(cor.matrix2$p), sig.level = 0.05, insig = "blank",
         title ="Inorganic elements", tl.srt = 45,
         tl.cex = 0.7, mar=c(0,0,1,0)) # significance level p<0.05

```

Inorganic elements



SAVING TABLE with significant DEGs in each exposure group

```
#-----#
blank_excel <- createWorkbook()

Map(function(df, tab_name){

  addWorksheet(blank_excel, tab_name)
  writeData(blank_excel, tab_name, df)
},

b4, names(b4)
)

## $CBF
## [1] 0
##
## $CBS
## [1] 0
##
## $PLF
## [1] 0
##
## $PLS
## [1] 0
```

```
##  
## $PWF  
## [1] 0  
##  
## $PWS  
## [1] 0  
  
saveWorkbook(blank_excel, file = "Table E1.xlsx", overwrite = TRUE)
```