Master 2 BioInformatique Modélisation et Statistique (BIMS), Université de Rouen Normandie

Functional Enrichment Analysis - Development of a Shiny application

RNA DAiSY seq

Differential expression Analysis In ShinY for RNAseq

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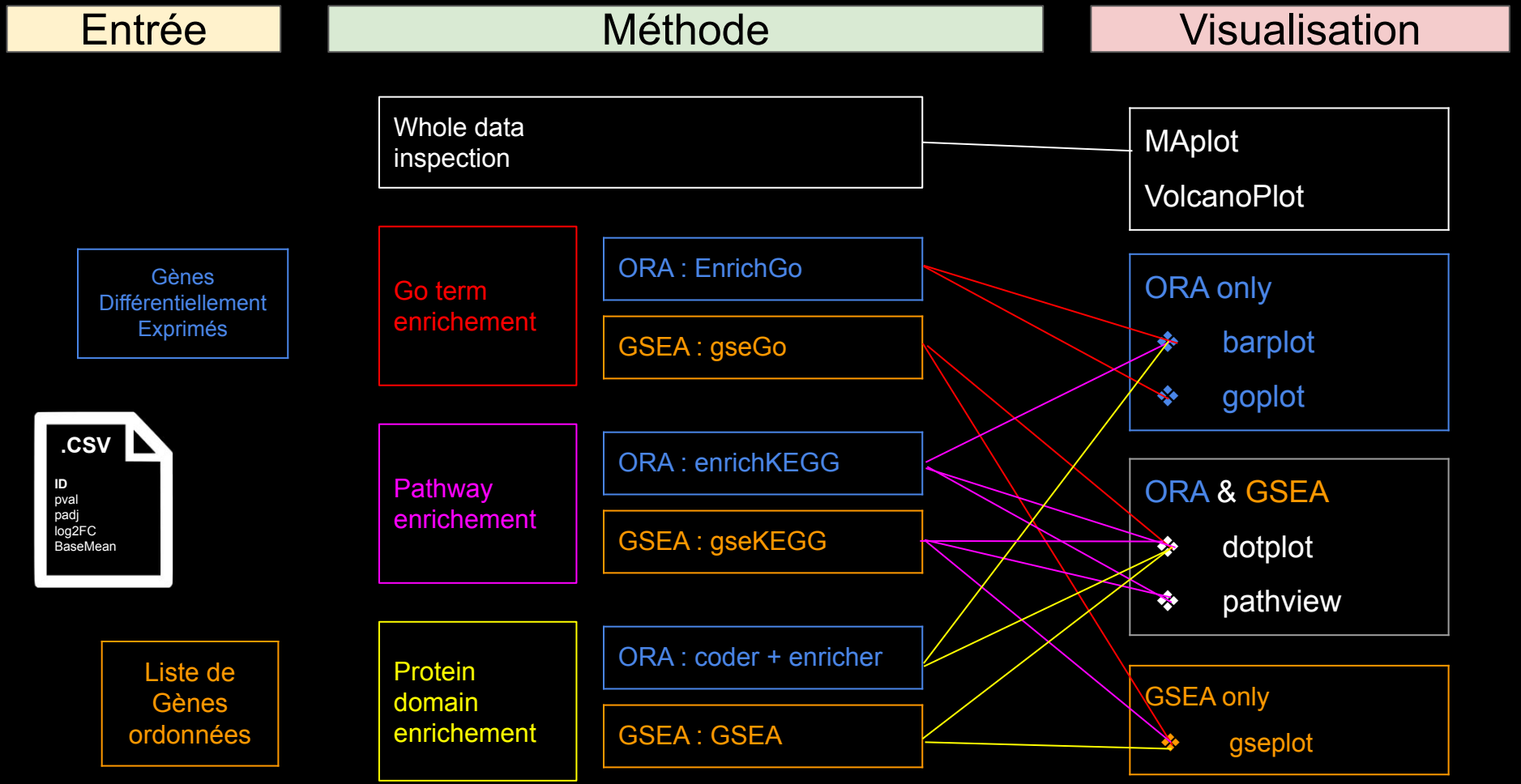
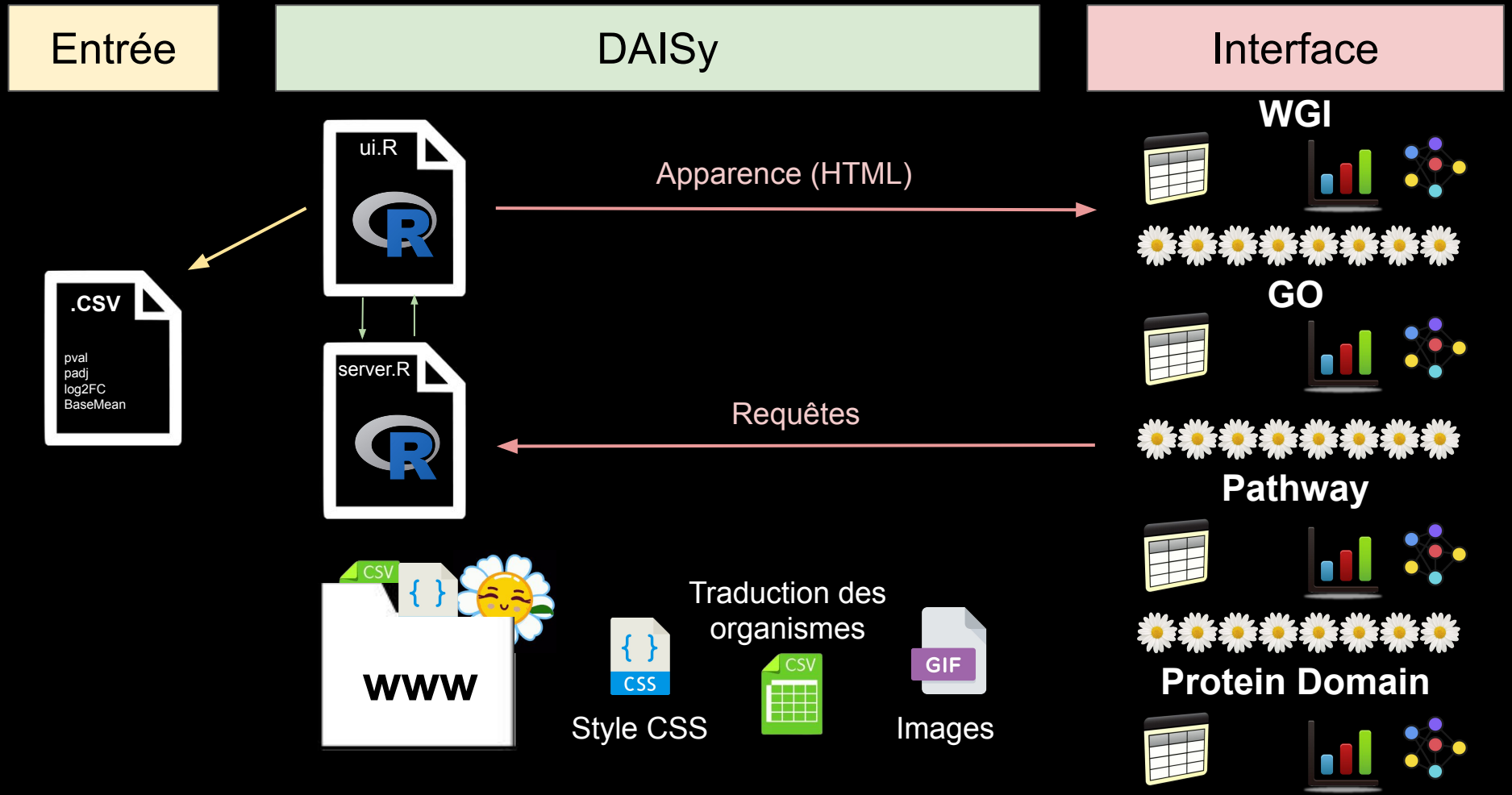

Entrée
Méthode
Visualisation
Whole data inspection
MAplot
VolcanoPlot
Gènes Différentiellement Exprimés
Go term enrichement
ORA : EnrichGo
GSEA : gseGo
ORA only
barplot
goplot
.csv
ID
pval
padj
log2FC
BaseMean
Pathway enrichement
ORA : enrichKEGG
GSEA : gseKEGG
ORA & GSEA
dotplot
pathview
Liste de Gènes ordonnées
Protein domain enrichement
ORA : coder + enricher
GSEA : GSEA
GSEA only
gseplot

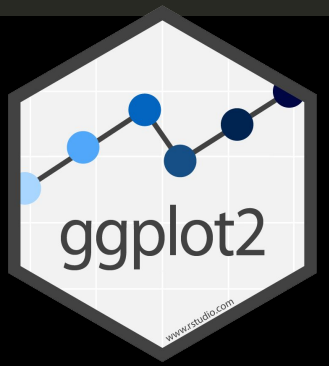

Entrée
DAISy
Interface
ui.R
server.R
.csv
pval
padj
log2FC
BaseMean
Apparence (HTML)
Requêtes
WGI
GO
Pathway
Protein Domain
CSV
{ }
www
CSS
Style CSS
Traduction des organismes
CSV
GIF
Images

Lancement de la démonstration

Whole genome inspection

```
re <- reactive({
        file <- input$file1
        ext <- tools::file_ext(file$datapath)
        req(file)
        validate(need(ext == "csv", "Invalid file. Please upload a .csv file"))
        data <- read.csv(file$datapath, header = TRUE, sep = ";")
        data <- na.omit(data)
        required_columns <- c("ID", "baseMean", "log2FC", "pval", "padj")
        column_names <- colnames(data)
        shiny::validate(need(all(required_columns %in% column_names), "Missing required columns"))
        data
})
```

```
p <- ggplot(
      data = data_plot_plotly,
      mapping = aes(x = log2FC, y=-log10(padj), col=diffexpressed, key = key)) +
      geom_point(size = 0.45) +
      theme_bw()  +
      scale_color_manual(values=c("red", "black", "green")) +
      xlab("log2 fold change") +
      ylab("-log10(p-value)")
```



Gene Ontology enrichement

```
goGse_annot <-  eventReactive(input$Run_Annotation_go, if(input$method_go == 2){
      espece_id <- espece_id()
      orga_translate_table <- orga_translate_table()
      go <- re()
      original_gene_list <- go$log2FC
      names(original_gene_list) <- go$ID
      gene_list<-na.omit(original_gene_list)
      gene_list <- sort(gene_list, decreasing=TRUE)

      gse <- gseGO(geneList=gene_list,
                   ont = input$Ontology,
                   keyType = 'ENSEMBL',
                   pvalueCutoff = input$pvalue_go,
                   minGSSize = 3,
                   maxGSSize = 800,
                   verbose = TRUE,
                   OrgDb = orga_translate_table[espece_id,2],
                   pAdjustMethod = input$Ajustement_go)

})
```

GSEA

```
goGse_enrich <-  eventReactive(input$Run_Annotation_go, if(input$method_go == 1){
      espece_id <- espece_id()
      orga_translate_table <- orga_translate_table()
      go <- re()
      original_gene_list <- go$log2FC
      names(original_gene_list) <- go$ID
      gene_list<-na.omit(original_gene_list)
      gene_list = sort(gene_list, decreasing = TRUE)
      sig_genes_df = subset(go, padj < input$pvalue_go)
      genes <- sig_genes_df$log2FC
      names(genes) <- sig_genes_df$ID
      genes <- na.omit(genes)
      tresholdLog2FoldChange <- tresholdLog2FoldChange()

      if (input$type_go == "over"){
        genes <- names(genes)[genes > tresholdLog2FoldChange]
      }
      else if(input$type_go == "under") {
        genes <- names(genes)[genes < -tresholdLog2FoldChange]
      }
      else if(input$type_go == "both") {
        genes <- names(genes)[abs(genes) > tresholdLog2FoldChange]
      }
      go_enrich <- enrichGO(gene = genes,
                            universe = names(gene_list),
                            OrgDb = orga_translate_table[espece_id,2],
                            keyType = 'ENSEMBL',
                            readable = T,
                            ont = input$Ontology,
                            pvalueCutoff = input$pvalue_go,
                            pAdjustMethod = input$Ajustement_go
                            )
    })
```

ORA

KEGG, Pathway enrichement

```
kegg_data <- eventReactive(input$Run_Pathway, {
      data <- re()
      espece_id <- espece_id()
      orga_translate_table <- orga_translate_table()
      adj_method <- input$kegg_adj_method

      ids = bitr(data$ID, fromType = "ENSEMBL", toType = "ENTREZID",
                 OrgDb=orga_translate_table[espece_id,2])

      dedup_ids = ids[!duplicated(ids[c("ENSEMBL")]),]
      df2 = data[data$ID %in% dedup_ids$ENSEMBL,]

      df2$Y = dedup_ids$ENTREZID

      kegg_gene_list <- df2$log2FC
      names(kegg_gene_list) <- df2$Y

      kegg_gene_list<-na.omit(kegg_gene_list)

      kegg_gene_list = sort(kegg_gene_list, decreasing = TRUE)

GSEA  if(input$method == 2){
        res <- gse_kegg(kegg_gene_list, adj_method, orga_translate_table[espece_id,])
      }
ORA   if(input$method == 1){
        res <- ora_kegg(df2, kegg_gene_list, adj_method, orga_translate_table[espece_id,])
      }
      list(res =res, kegg_gene_list=kegg_gene_list)
})
```

```
gse_kegg <- function(kegg_gene_list, adj_method, orga_translate_table) {
    if (input$db == 1){
    gse_result <- gseKEGG(geneList     = kegg_gene_list,
                          organism     = orga_translate_table[1,4],
                          nPerm        = 10000,
                          minGSSize    = 3,
                          maxGSSize    = 800,
                          pvalueCutoff = input$pvalue_gsea,
                          pAdjustMethod = adj_method,
                          keyType      = "ncbi-geneid")
    }
    else {
    gse_result <- gsePathway(geneList     = kegg_gene_list,
                             organism     = orga_translate_table[1,5],
                             nPerm        = 10000,
                             minGSSize    = 3,
                             maxGSSize    = 800,
                             pvalueCutoff = input$pvalue_gsea,
                             pAdjustMethod = adj_method)
    }
    return(gse_result)
}
```

KEGG

Reactome

GSEA

clusterProfiler

KEGG, Pathway enrichement

```r
kegg_data <- eventReactive(input$Run_Pathway, {
    data <- re()
    espece_id <- espece_id()
    orga_translate_table <- orga_translate_table()
    adj_method <- input$kegg_adj_method

    ids = bitr(data$ID, fromType = "ENSEMBL", toType = "ENTREZID",
               OrgDb=orga_translate_table[espece_id,2])

    dedup_ids = ids[!duplicated(ids[c("ENSEMBL")]),]
    df2 = data[data$ID %in% dedup_ids$ENSEMBL,]

    df2$Y = dedup_ids$ENTREZID

    kegg_gene_list <- df2$log2FC
    names(kegg_gene_list) <- df2$Y

    kegg_gene_list<-na.omit(kegg_gene_list)

    kegg_gene_list = sort(kegg_gene_list, decreasing = TRUE)

    if(input$method == 2){
      res <- gse_kegg(kegg_gene_list, adj_method, orga_translate_table[espece_id,])
    }
    if(input$method == 1){
      res <- ora_kegg(df2, kegg_gene_list, adj_method, orga_translate_table[espece_id,])
    }
    list(res =res, kegg_gene_list=kegg_gene_list)
})
```

GSEA

ORA

```r
ora_kegg <- function(df2, kegg_gene_list, adj_method, orga_translate_table) {
        # Exctract significant results from previous pvalue adjusted threshold
        kegg_sig_genes_df = subset(df2, padj < input$pvalue)
        # Vector of log2foldChange with ENTREZ IDs as names
        kegg_genes <- kegg_sig_genes_df$log2FC
        names(kegg_genes) <- kegg_sig_genes_df$Y
        kegg_genes <- na.omit(kegg_genes)
        # filter on log2fold change (under or over expressed DEG)
        if (input$type == 1){
          kegg_genes <- names(kegg_genes)[kegg_genes > input$tresholdLog2FoldChange]
        }
        else if(input$type == 2) {
          kegg_genes <- names(kegg_genes)[kegg_genes < - input$tresholdLog2FoldChange]
        }
        else {
          kegg_genes <- names(kegg_genes)[kegg_genes < - input$tresholdLog2FoldChange
                                          || kegg_genes > input$tresholdLog2FoldChange]
        }
        #Database to use for annotation
        if (input$db == 1){
          ora_result <- enrichKEGG(gene=kegg_genes, universe=names(kegg_gene_list),
                                   organism=orga_translate_table[1,4],
                                   pvalueCutoff = input$pvalue_gsea,
                                   keyType = "ncbi-geneid",
                                   pAdjustMethod = adj_method)
        }
        else {
          ora_result <- enrichPathway(gene=kegg_genes, universe=names(kegg_gene_list),
                                      organism=orga_translate_table[1,5],
                                      pvalueCutoff = input$pvalue_gsea,
                                      pAdjustMethod = adj_method)
        }
        return(ora_result)
}
```

KEGG

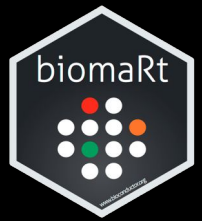
Reactome

ORA

clusterProfiler

Protein domain enrichement

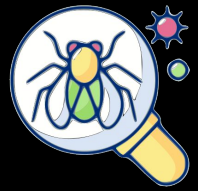
```
interpro_id_raw <- getBM(
  attributes=c('interpro', 'interpro_description', 'ensembl_transcript_id', 'ensembl_gene_id'),
  filters = 'ensembl_gene_id',
  values = resOrdered$ID,
  uniqueRows = FALSE,
  mart = ensembl)

create_table_enrichment = function(GeneList, GeneRef){

  Gene.Bg.ratio = get_Gene_and_Bg_ratio(GeneList = GeneList, GeneRef = GeneRef)
  Bg.ratio = signif(100 * Gene.Bg.ratio$m/(Gene.Bg.ratio$m + Gene.Bg.ratio$n), 3)
  Gene.ratio = signif(100 * Gene.Bg.ratio$x / Gene.Bg.ratio$k, 3)

  test = hypergeom_test(x = Gene.Bg.ratio$x,k = Gene.Bg.ratio$k,
                        m = Gene.Bg.ratio$m, n = Gene.Bg.ratio$n)

  table.enrich = data.frame(interpro_ID = Gene.Bg.ratio$Term,
                            pvalue = signif(test$pvalue_fisher.test, 3),
                            padj = signif(test$padj, 3),
                            BgRatio = Bg.ratio,
                            GeneRatio = Gene.ratio,
                            count = Gene.Bg.ratio$x,
                            )

return (table.enrich[order(table.enrich$pval), ])
```

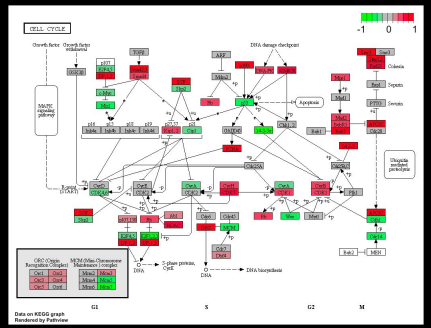


What next ?

Fichier d'entré :
trop spécifique (ENSEMBL)

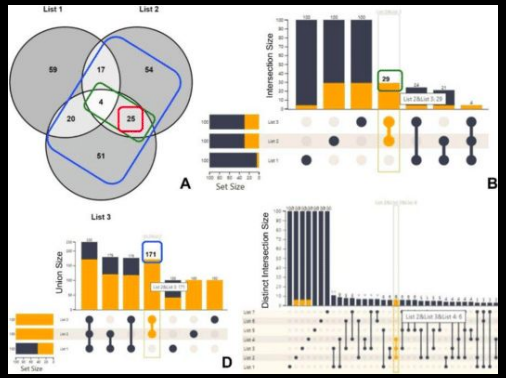
Nombre d'organismes modèles restreint …
et les autres ?



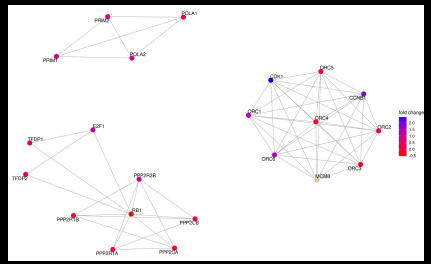


Pathview :
optimiser l'affichage
des réseaux

Plus de deux conditions
à comparer ?



https://www.researchgate.net/publication/352068166/figure/fig1/AS:1030430237151232@1622685121380/UpSet-plot-vs-Venn-diagrams-A-Intersection-of-the-three-gene-lists-100-genes-each.png



Reactome :
Obtenir un
réseau lisible
(viewPathway())

Merci de votre attention

Annotation fonctionnelle pour :

- GO
- KEGG
- Domaines Protéiques

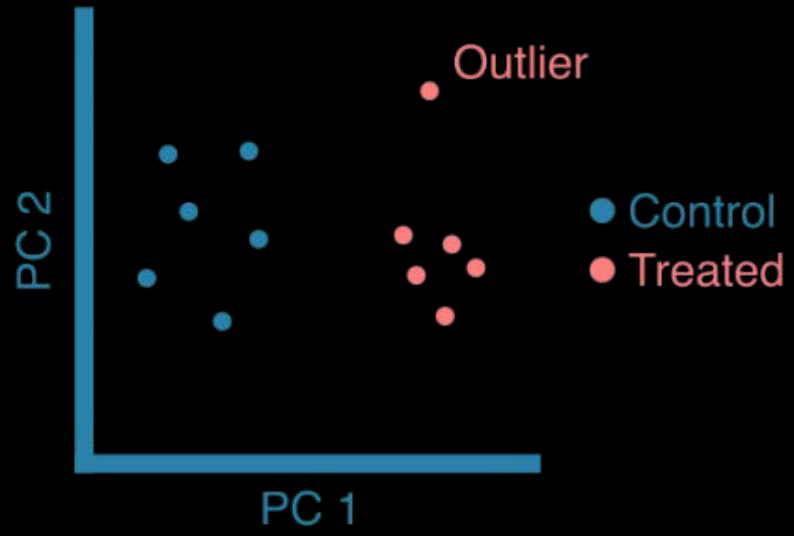
- Fichier d'entrée : trop spécifique (ENSEMBL)
- KEGG
  - Pathview : affichage des réseaux
  - Reactom : fonction viewPathway()

Organismes modèles… ok, et les autres ?

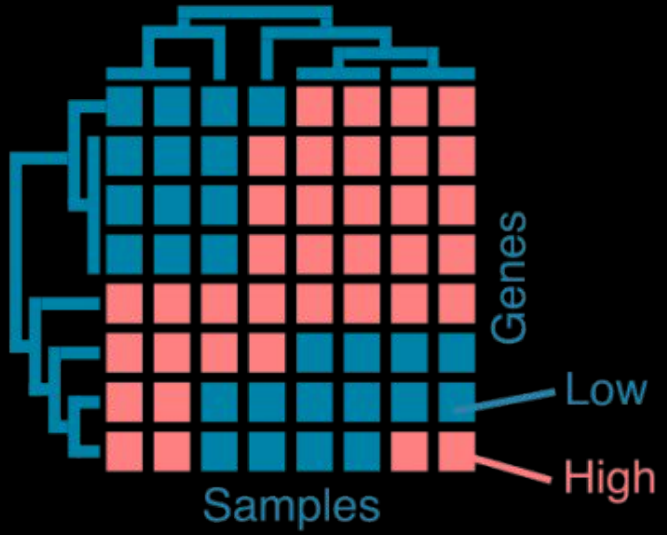
Restreint à un petit nombre d'organismes modèles

Enrichissement fonctionnel : souvent plus de deux conditions à comparer

Principal component analysis



Expression heatmap



GO Term Enrichement : Plots

server.R

```r
output$dotplot_gsea_go <-renderPlot({
      gse<-goGse_annot()
      require(DOSE)
      dotplot(gse, showCategory = input$showCategory_dotplot, title = "gsea dotplot" , split=".sign") + facet_grid(.~.sign)
    })

    output$ridgeplot_go<-renderPlot({
      gse<-goGse_annot()
      require(DOSE)
      ridgeplot(gse, showCategory =input$showCategory_ridgeplot)
    })

    output$gsea_plot_go <-renderPlot({
      gse<-goGse_annot()
      require(DOSE)
      gseaplot2(gse, geneSetID = input$showCategory_gseaplot)
})
```

uii.R

```r
conditionalPanel(

  condition = "input.method_go == 2",

  box(title = "Dot Plot GSEA", solidHeader = T, status = "success", width = 12, collapsible = T,id = "dotplot",
      fluidRow(
          column(3,
              wellPanel(
                  numericInput("showCategory_dotplot", "number of categories to show", value = 5))),
          column(9,
              wellPanel(
                  shinycustomloader::withLoader(plotlyOutput("dotplot_gsea_go", height = "450px"), type = "image", loader = "wait.gif")
```

GO Term Enrichement : Table

server.R

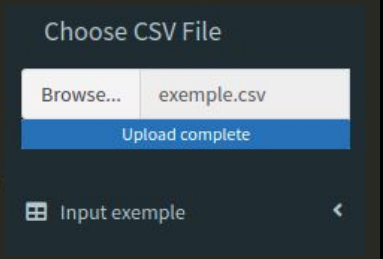
```
output$go_enrich_table_test <- DT::renderDataTable(DT::datatable({
      if (input$method_go == 1){
        data <- as.data.frame(goGse_enrich() )%>%
        mutate(interpro_link = paste0("<a href='https://amigo.geneontology.org/amigo/term/", Description,"' target='_blank'>", Description,"</a>"))
        col_a_afficher = c("URL",
                           "Description",
                           "GeneRatio",
                           "BgRatio",
                           "pvalue",
                           "p.adjust",
                           "qvalue")
        data[col_a_afficher]
      }
      else if (input$method_go == 2){
        data <- as.data.frame(goGse_annot() )%>%
        mutate(interpro_link = paste0("<a href='https://amigo.geneontology.org/amigo/term/", Description,"' target='_blank'>", Description,"</a>"))
        col_a_afficher = c("URL",
                           "Description",
                           "enrichmentScore",
                           "pvalue",
                           "p.adjust",
                           "rank")
        data[col_a_afficher]
      }
      updateSelectInput(session,"paths")
      DT::datatable(data)
      },
escape = FALSE))

```

uii.R

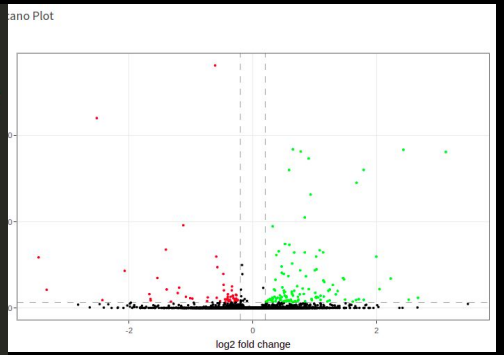
```
box (
  shinycustomloader::withLoader(dataTableOutput("go_enrich_table"), type = "image", loader = "wait.gif"),
width = 12) ,
```

Annexes

```
re <- reactive({
        file <- input$file1
        ext <- tools::file_ext(file$datapath)
        req(file)
        validate(need(ext == "csv", "Invalid file. Please upload a .csv file"))
        data <- read.csv(file$datapath, header = TRUE, sep = ";")
        data <- na.omit(data)
        required_columns <- c("ID", "baseMean", "log2FC", "pval", "padj")
        column_names <- colnames(data)
        shiny::validate(need(all(required_columns %in% column_names), "Missing required columns"))
        data
})
```

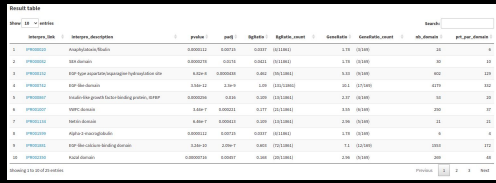


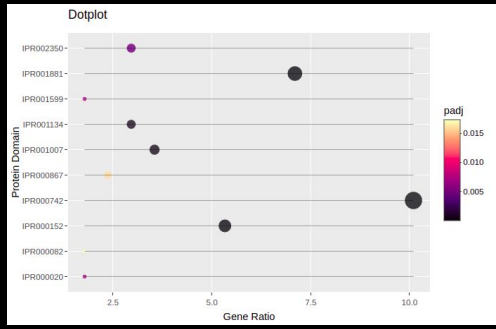

```
p <- ggplot(
      data = data_plot_plotly,
      mapping = aes(x = log2FC, y=-log10(padj), col=diffexpressed, key = key)) +
      geom_point(size = 0.45) +
      theme_bw()  +
      scale_color_manual(values=c("red", "black", "green")) +
      xlab("log2 fold change") +
      ylab("-log10(p-value)")
```

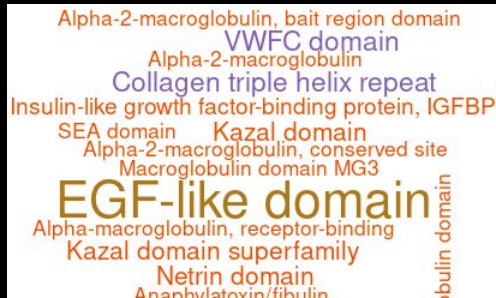


Enrichissement en domaines protéiques

```
interpro_id_raw <- getBM(
  attributes=c('interpro', 'interpro_description', 'ensembl_transcript_id', 'ensembl_gene_id'),
  filters = 'ensembl_gene_id',
  values = resOrdered$ID,
  uniqueRows = FALSE,
  mart = ensembl)

create_table_enrichment = function(GeneList, GeneRef){

  Gene.Bg.ratio = get_Gene_and_Bg_ratio(GeneList = GeneList, GeneRef = GeneRef)
  Bg.ratio = signif(100 * Gene.Bg.ratio$m/(Gene.Bg.ratio$m + Gene.Bg.ratio$n), 3)
  Gene.ratio = signif(100 * Gene.Bg.ratio$x / Gene.Bg.ratio$k, 3)

  test = hypergeom_test(x = Gene.Bg.ratio$x,k = Gene.Bg.ratio$k,
                        m = Gene.Bg.ratio$m, n = Gene.Bg.ratio$n)

  table.enrich = data.frame(interpro_ID = Gene.Bg.ratio$Term,
                            pvalue = signif(test$pvalue_fisher.test, 3),
                            padj = signif(test$padj, 3),
                            BgRatio = Bg.ratio,
                            GeneRatio = Gene.ratio,
                            count = Gene.Bg.ratio$x,
                            )

return (table.enrich[order(table.enrich$pval), ])
```







Procédure [Irizarry *et al.*, 2009, Mootha *et al.*, 2003, Subramanian *et al.*, 2005]

- Soit un ensemble de gènes S pré-défini (ex : Terme GO)

1. Calcul d'un score d'enrichissement (Y et N sont de même signe)
   - Gènes sont ordonnés (par p-value, par LFC ou autre)
   - Score calculé :

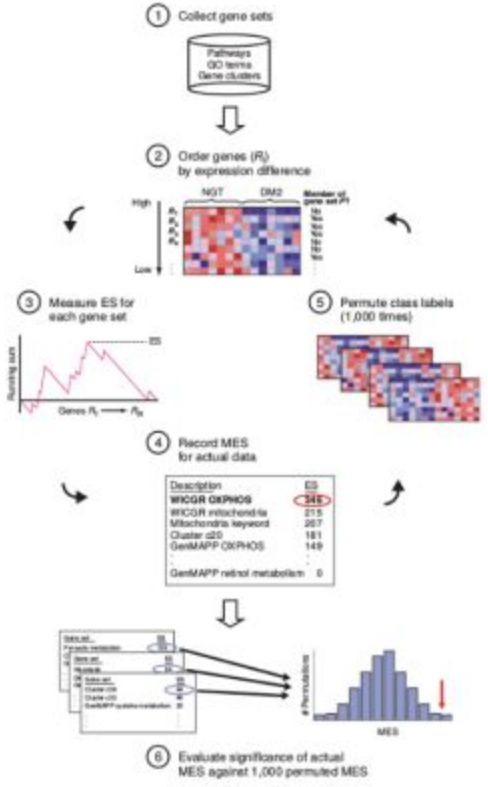
$$S_{c_i} = \begin{cases} +Y & \text{si le gène} i \in S \\ -N & \text{sinon.} \end{cases}$$

$$ES = \max_{1 \le i \le n} \sum_{i=1}^{j} S_{c_i} \text{ si } Y > 0 \text{ ou } ES = \min_{1 \le i \le n} \sum_{i=1}^{j} S_{c_i} \text{ si } Y < 0$$

2. Estimation du niveau de significativité du score ES
   - Permutation de l'ensemble S (1 000)
   - Calcul du score ES pour les ensembles permutés qu'on stocke
3. Comparaison du score ES calculé à celui des ensembles permutés
   - P-valeur calculée :

$$p = \frac{1}{1000} \sum_{j=1}^{1000} \mathbf{1}_{ES \ge ES_j} \text{ si } Y > 0 \text{ ou } p = \frac{1}{1000} \sum_{j=1}^{1000} \mathbf{1}_{ES \le ES_j} \text{ si } Y < 0$$

Étapes



Mootha *et al.*, 2003

Résumé

- Test de randomisation/Kolmogorov-Smirnov
  - Détermine si un échantillon suit bien une loi donnée
  - Hypothèses sont :

$$H_0 : D_1 = D_2 \quad \text{vs} \quad H_1 : D_1 \neq D_2$$

  - Hypothèse nulle : si l'enrichissement est présent, cela est dû au hasard

3 App 1: Connecting `ui` and `server`