BNM Project

Disease: Multiple Sclerosis

Project Overview:

This project analyzes Multiple Sclerosis using network medicine approaches including:

1. Building interactomes (BioGRID, HuRI, STRING, Reactome)
2. Disease gene prioritization (DIAMOnD and Diffusion)
3. Functional enrichment analysis
4. Drug-gene interaction analysis
5. PROCONSUL comparison

Part 1: Interactome Construction

Setup and Configuration

```r
ensure_packages <- function(pkgs) {
  for (p in pkgs) {
    if (!requireNamespace(p, quietly = TRUE)) {
      install.packages(p, dependencies = TRUE)
    }
    suppressPackageStartupMessages(
      library(p, character.only = TRUE)
    )
  }
}
ensure_packages(c(
  "tidyverse", "igraph", "readr", "stringr", "tidyr", "Matrix", "biomaRt",
  "ggplot2", "reshape2"
))

# Bioconductor packages (clusterProfiler/org.Hs.eg.db/enrichplot/ReactomePA)
if (!requireNamespace("BiocManager", quietly = TRUE)) install.packages("BiocManager")

bioc_pkgs <- c("clusterProfiler", "org.Hs.eg.db", "enrichplot", "ReactomePA")
for (p in bioc_pkgs) {
  if (!requireNamespace(p, quietly = TRUE)) BiocManager::install(p, ask = FALSE, update = FALSE)
  library(p, character.only = TRUE)
}
```

```r
# User paths (EDIT THESE)
PATH_STRING_LINKS   <- "9606.protein.links.v12.0 STRING.txt.txt"
PATH_REACTOME_FI    <- "FIsInGene_04142025_with_annotations_REACTOME.txt"
PATH_BIOGRID_TAB3   <- "BIOGRID-ORGANISM-Homo_sapiens-5.0.252.tab3.txt"
PATH_HURI_2COL      <- "HuRI.tsv"
PATH_GEDIPNET_DISEASE <- "diseases_"
```

```r
OUTDIR <- "PART1_outputs"
dir.create(OUTDIR, showWarnings = FALSE, recursive = TRUE)
```

Helper Functions

```r
# Deduplicate an undirected edge list and remove self-loops
dedup_undirected_edges <- function(df, from = "from", to = "to") {
  df %>%
    dplyr::select(from = all_of(from), to = all_of(to)) %>%
    mutate(from = as.character(from), to = as.character(to)) %>%
    filter(!is.na(from), !is.na(to), from != "", to != "") %>%
    filter(from != to) %>%
    mutate(node_min = pmin(from, to), node_max = pmax(from, to)) %>%
    distinct(node_min, node_max) %>%
    transmute(from = node_min, to = node_max)
}
```

```r
# Build graph and extract LCC
graph_lcc <- function(edge_df, directed = FALSE) {
  g <- igraph::graph_from_data_frame(edge_df, directed = directed)
  comp <- igraph::components(g)
  giant_id <- which.max(comp$csize)
  nodes_lcc <- igraph::V(g)[comp$membership == giant_id]
  igraph::induced_subgraph(g, nodes_lcc)
}
```

```r
# Robust Ensembl connection (mirrors)
connect_ensembl <- function() {
  mirrors <- c("www", "useast", "uswest", "asia")
  for (m in mirrors) {
    message("Connecting to Ensembl mirror: ", m)
    out <- try(
      biomaRt::useEnsembl(biomart="ensembl", dataset="hsapiens_gene_ensembl", mirror=m),
      silent = TRUE
    )
    if (!inherits(out, "try-error")) return(out)
  }
  stop("Could not connect to any Ensembl mirror. Try later or change network/VPN.")
}
```

```r
# Map ENSP -> HGNC (STRING)
map_ensp_to_hgnc <- function(ensp_vec, mart) {
  ensp_vec <- unique(ensp_vec[!is.na(ensp_vec) & ensp_vec != ""])
  if (length(ensp_vec) == 0) return(tibble(ensembl_peptide_id = character(), hgnc_symbol = character())
  biomaRt::getBM(
    attributes = c("ensembl_peptide_id", "hgnc_symbol"),
    filters    = "ensembl_peptide_id",
    values     = ensp_vec,
    mart       = mart
  ) %>% as_tibble()
}
```

```r
# Map ENSG -> HGNC (HuRI)
map_ensg_to_hgnc <- function(ensg_vec, mart) {
  ensg_vec <- unique(ensg_vec[!is.na(ensg_vec) & ensg_vec != ""])
  if (length(ensg_vec) == 0) return(tibble(ensembl_gene_id = character(), hgnc_symbol = character()))
  biomaRt::getBM(
    attributes = c("ensembl_gene_id", "hgnc_symbol"),
    filters    = "ensembl_gene_id",
    values     = ensg_vec,
    mart       = mart
  ) %>% as_tibble()
}
```

```r
# Parse GeDiPNet "Associated genes" robustly
read_seed_genes_gedipnet <- function(path, col = "Associated genes") {
  df <- readr::read_delim(path, delim = "\t", show_col_types = FALSE)
  if (!(col %in% names(df))) {
    stop("Column '", col, "' not found. Columns are: ", paste(names(df), collapse = ", "))
  }

  seeds <- df %>%
    dplyr::select(all_of(col)) %>%
    mutate(across(everything(), as.character)) %>%
    tidyr::separate_rows(all_of(col), sep = "[,;\\s]+") %>%
    mutate(gene = stringr::str_trim(.data[[col]])) %>%
    filter(!is.na(gene), gene != "") %>%
    distinct(gene) %>%
    pull(gene)

  seeds
}
```

```r
# Optional: HGNC symbol checking
hgnc_check <- function(symbols) {
  symbols <- unique(symbols)
  if (!requireNamespace("HGNChelper", quietly = TRUE)) {
    return(list(final = symbols, report = tibble(Symbol = symbols, Approved = NA)))
  }
  chk <- HGNChelper::checkGeneSymbols(symbols)
  final <- ifelse(is.na(chk$Suggested.Symbol) | chk$Suggested.Symbol == "", chk$x, chk$Suggested.Symbol
  report <- tibble(
    Symbol = chk$x,
    Approved = chk$Approved,
    Suggested = chk$Suggested.Symbol
  )
  list(final = unique(final), report = report)
}
```

```r
# Table 1 row builder
tab1_row <- function(g_lcc, seed_genes, name) {
  node_names <- igraph::V(g_lcc)$name
  seeds_present <- intersect(seed_genes, node_names)

  g_dis <- igraph::induced_subgraph(g_lcc, seeds_present)

  dis_lcc_size <- 0L
  if (igraph::vcount(g_dis) > 0 && igraph::ecount(g_dis) > 0) {
    comp <- igraph::components(g_dis)
    giant_id <- which.max(comp$csize)
    g_dis_lcc <- igraph::induced_subgraph(g_dis, igraph::V(g_dis)[comp$membership == giant_id])
    dis_lcc_size <- igraph::vcount(g_dis_lcc)
  } else if (igraph::vcount(g_dis) > 0) {
    dis_lcc_size <- 1L
  }

  tibble(
    Interactome = name,
    Nodes_LCC = igraph::vcount(g_lcc),
    Links_LCC = igraph::ecount(g_lcc),
    DiseaseGenes_Present = length(seeds_present),
    DiseaseGenes_Percent = round(100 * length(seeds_present) / length(seed_genes), 2),
    DiseaseInteractome_LCC_Size = dis_lcc_size
  )
}
```

Build Interactomes

```r
mart <- connect_ensembl()
```

STRING Interactome

```r
message("Building STRING interactome...")
string_raw <- readr::read_delim(
  PATH_STRING_LINKS,
  delim = " ",
  trim_ws = TRUE,
  show_col_types = FALSE
)

stopifnot(all(c("protein1", "protein2") %in% names(string_raw)))

string_ensp <- string_raw %>%
  transmute(
    from_ensp = stringr::str_remove(as.character(protein1), "^9606\\."),
    to_ensp   = stringr::str_remove(as.character(protein2), "^9606\\.")
  ) %>%
  filter(from_ensp != to_ensp)

all_ensp <- unique(c(string_ensp$from_ensp, string_ensp$to_ensp))
map_string <- map_ensp_to_hgnc(all_ensp, mart)

string_mapped <- string_ensp %>%
  left_join(map_string, by = c("from_ensp" = "ensembl_peptide_id")) %>%
  dplyr::rename(from_symbol = hgnc_symbol) %>%
  left_join(map_string, by = c("to_ensp" = "ensembl_peptide_id")) %>%
  dplyr::rename(to_symbol = hgnc_symbol) %>%
  filter(!is.na(from_symbol), from_symbol != "",
         !is.na(to_symbol),   to_symbol   != "")

string_edges <- dedup_undirected_edges(string_mapped %>% transmute(from = from_symbol, to = to_symbol))
```

```r
g_string_lcc <- graph_lcc(string_edges)

message("STRING LCC nodes: ", vcount(g_string_lcc), " | edges: ", ecount(g_string_lcc))
```

Reactome Interactome

```r
message("Building Reactome interactome...")
reactome_raw <- readr::read_tsv(PATH_REACTOME_FI, show_col_types = FALSE)

stopifnot(all(c("Gene1", "Gene2") %in% names(reactome_raw)))

reactome_edges <- dedup_undirected_edges(
  reactome_raw %>% transmute(from = Gene1, to = Gene2)
)
g_reactome_lcc <- graph_lcc(reactome_edges)

message("Reactome LCC nodes: ", vcount(g_reactome_lcc), " | edges: ", ecount(g_reactome_lcc))
```

BioGRID Interactome

```r
message("Building BioGRID interactome...")
biogrid_raw <- readr::read_tsv(PATH_BIOGRID_TAB3, show_col_types = FALSE)

required_cols_bg <- c(
  "Organism ID Interactor A", "Organism ID Interactor B",
  "Experimental System Type",
  "Official Symbol Interactor A", "Official Symbol Interactor B"
)
stopifnot(all(required_cols_bg %in% names(biogrid_raw)))

biogrid_edges <- biogrid_raw %>%
  filter(`Organism ID Interactor A` == 9606,
         `Organism ID Interactor B` == 9606) %>%
  filter(`Experimental System Type` == "physical") %>%
  transmute(from = `Official Symbol Interactor A`,
            to   = `Official Symbol Interactor B`)

biogrid_edges <- dedup_undirected_edges(biogrid_edges)
g_biogrid_lcc <- graph_lcc(biogrid_edges)

message("BioGRID LCC nodes: ", vcount(g_biogrid_lcc), " | edges: ", ecount(g_biogrid_lcc))
```

HuRI Interactome

```r
message("Building HuRI interactome...")
huri_raw <- readr::read_tsv(PATH_HURI_2COL, col_names = c("from", "to"), show_col_types = FALSE)

huri_raw <- huri_raw %>%
  mutate(from = as.character(from), to = as.character(to)) %>%
  filter(!is.na(from), !is.na(to), from != "", to != "") %>%
  filter(str_detect(from, "^ENSG"), str_detect(to, "^ENSG"))
```

```r
all_ensg <- unique(c(huri_raw$from, huri_raw$to))
map_huri <- map_ensg_to_hgnc(all_ensg, mart)

huri_mapped <- huri_raw %>%
  left_join(map_huri, by = c("from" = "ensembl_gene_id")) %>%
  dplyr::rename(from_symbol = hgnc_symbol) %>%
  left_join(map_huri, by = c("to" = "ensembl_gene_id")) %>%
  dplyr::rename(to_symbol = hgnc_symbol) %>%
  filter(!is.na(from_symbol), from_symbol != "",
         !is.na(to_symbol),   to_symbol   != "")

huri_edges <- dedup_undirected_edges(huri_mapped %>% transmute(from = from_symbol, to = to_symbol))
g_huri_lcc <- graph_lcc(huri_edges)

message("HuRI LCC nodes: ", vcount(g_huri_lcc), " | edges: ", ecount(g_huri_lcc))
```

```r
# Bundle interactomes
interactomes_lcc <- list(
  BioGRID  = g_biogrid_lcc,
  HuRI     = g_huri_lcc,
  STRING   = g_string_lcc,
  Reactome = g_reactome_lcc
)
```

Load Disease Genes

```r
message("Reading seed genes from GeDiPNet...")
seed_genes_raw <- read_seed_genes_gedipnet(PATH_GEDIPNET_DISEASE, col = "Associated genes")
message("Parsed seed genes: ", length(seed_genes_raw))

chk <- hgnc_check(seed_genes_raw)
seed_genes <- chk$final

if (!is.null(chk$report)) {
  write_csv(chk$report, file.path(OUTDIR, "HGNC_check_report.csv"))
  message("Saved HGNC check report")
}

message("Final seed genes after optional correction: ", length(seed_genes))
```

Table 1: Interactome Summary

```r
message("Building Table 1...")
table1 <- bind_rows(lapply(names(interactomes_lcc), function(nm) {
  tab1_row(interactomes_lcc[[nm]], seed_genes, nm)
}))

write_csv(table1, file.path(OUTDIR, "TABLE1_interactome_summary.csv"))
print(table1)
```

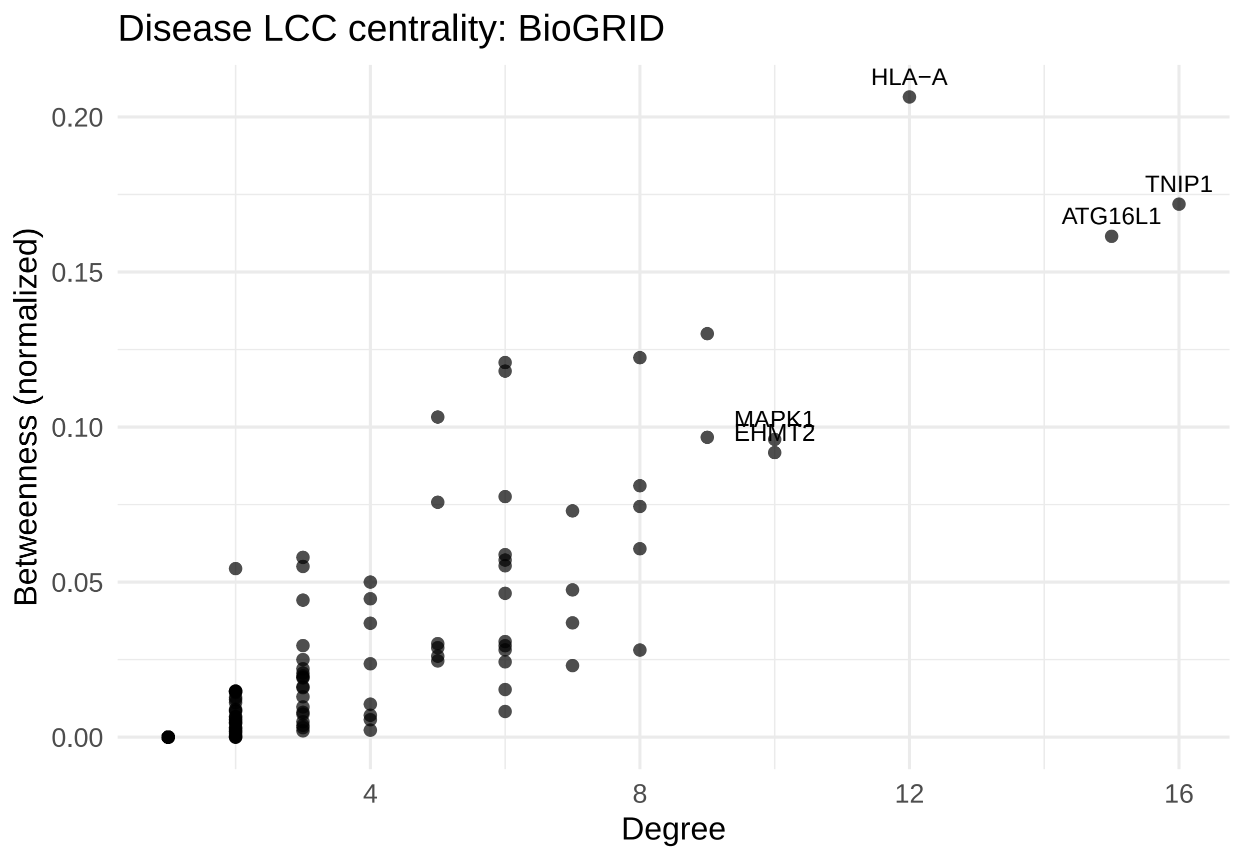
```
## # A tibble: 4 x 6
##   Interactome Nodes_LCC Links_LCC DiseaseGenes_Present DiseaseGenes_Percent
##   <chr>           <dbl>     <dbl>                <int>                <dbl>
## 1 BioGRID         20182    907435                   247                   84.3
## 2 HuRI             8938     62778                   112                   38.2
## 3 STRING          18920   6566519                   239                   81.6
## 4 Reactome        13262    272298                   202                   68.9
## # i 1 more variable: DiseaseInteractome_LCC_Size <dbl>
```

Table 2

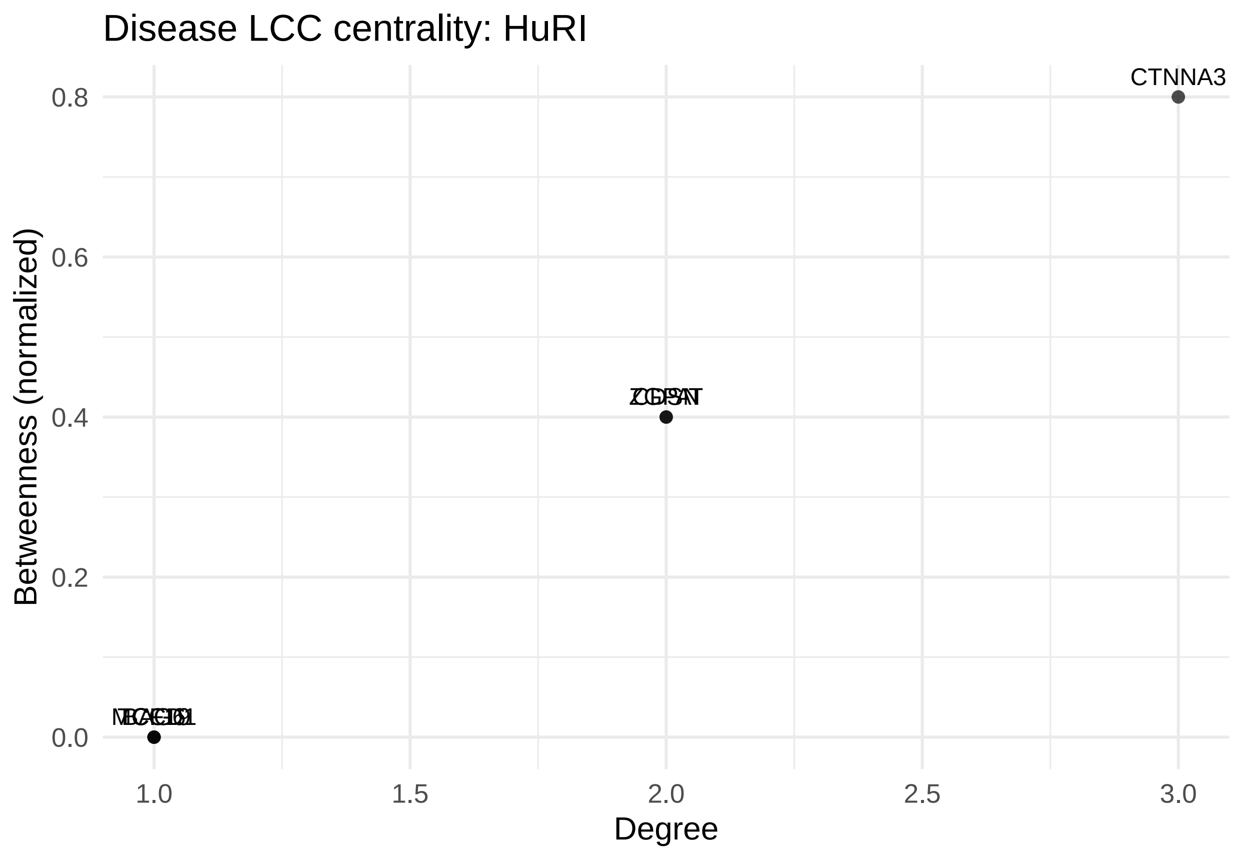
```
# ----------------------------------------------------------------------
# Table 2: Disease LCC centrality metrics (ALL interactomes)
# ----------------------------------------------------------------------

OUT2B <- "PART1_outputs/table2_centrality"
dir.create(OUT2B, showWarnings = FALSE, recursive = TRUE)

table2_results <- list()
table2_top20   <- list()

for (net_name in names(interactomes_lcc)) {

  message("Computing Table 2 for interactome: ", net_name)

  g <- interactomes_lcc[[net_name]]

  # Disease genes present in this interactome LCC
  seeds_present <- intersect(seed_genes, V(g)$name)

  if (length(seeds_present) == 0) {
    warning("No disease genes present in ", net_name, " LCC. Skipping.")
    next
  }

  # Disease subgraph
  g_disease <- induced_subgraph(g, seeds_present)

  if (vcount(g_disease) == 0 || ecount(g_disease) == 0) {
    warning("Disease subgraph empty for ", net_name, ". Skipping.")
    next
  }

  # Disease LCC
  comp_dis <- components(g_disease)
  giant_id <- which.max(comp_dis$csize)
  g_disease_lcc <- induced_subgraph(
    g_disease,
    V(g_disease)[comp_dis$membership == giant_id]
  )

  message(
    "  Disease LCC nodes=", vcount(g_disease_lcc),
    " edges=", ecount(g_disease_lcc)
  )

  # Centrality metrics
```

```r
centrality_tbl <- tibble(
  Interactome = net_name,
  Gene_name   = V(g_disease_lcc)$name,
  Degree      = degree(g_disease_lcc),
  Betweenness = betweenness(g_disease_lcc, directed = FALSE, normalized = TRUE),
  Eigenvector = eigen_centrality(g_disease_lcc)$vector,
  Closeness   = closeness(g_disease_lcc, normalized = TRUE)
) %>%
  mutate(
    ratio_Betw_Degree = ifelse(Degree > 0, Betweenness / Degree, NA_real_)
  ) %>%
  arrange(desc(Degree), desc(Betweenness))

# Save full Table 2
write_csv(
  centrality_tbl,
  file.path(OUT2B, paste0("TABLE2_centrality_all_", net_name, ".csv"))
)

# Top-20 by degree
top20_tbl <- centrality_tbl %>% slice_head(n = 20)
print(top20_tbl)
write_csv(
  top20_tbl,
  file.path(OUT2B, paste0("TABLE2_top20_", net_name, ".csv"))
)

table2_results[[net_name]] <- centrality_tbl
table2_top20[[net_name]]   <- top20_tbl

# Scatter plot: Degree vs Betweenness
p <- ggplot(centrality_tbl, aes(x = Degree, y = Betweenness)) +
  geom_point(alpha = 0.7) +
  geom_text(
    data = centrality_tbl %>% slice_max(Degree, n = 5),
    aes(label = Gene_name),
    vjust = -0.7,
    size = 3
  ) +
  theme_minimal(base_size = 12) +
  labs(
    title = paste0("Disease LCC centrality: ", net_name),
    x = "Degree",
    y = "Betweenness (normalized)"
  )
ggplot(centrality_tbl, aes(x = Degree, y = Betweenness)) +
  geom_point(alpha = 0.7) +
  geom_text(
    data = centrality_tbl %>% slice_max(Degree, n = 5),
    aes(label = Gene_name),
    vjust = -0.7,
    size = 3
  ) +
    theme_minimal(base_size = 12) +
    labs(
      title = paste0("Disease LCC centrality: ", net_name),
      x = "Degree",
      y = "Betweenness (normalized)"
    )
  ggsave(
    file.path(OUT2B, paste0("FIG_table2_scatter_degree_betweenness_", net_name, ".png")),
    p,
    width = 9,
    height = 6,
    dpi = 300
  )

  print(p)
}
```

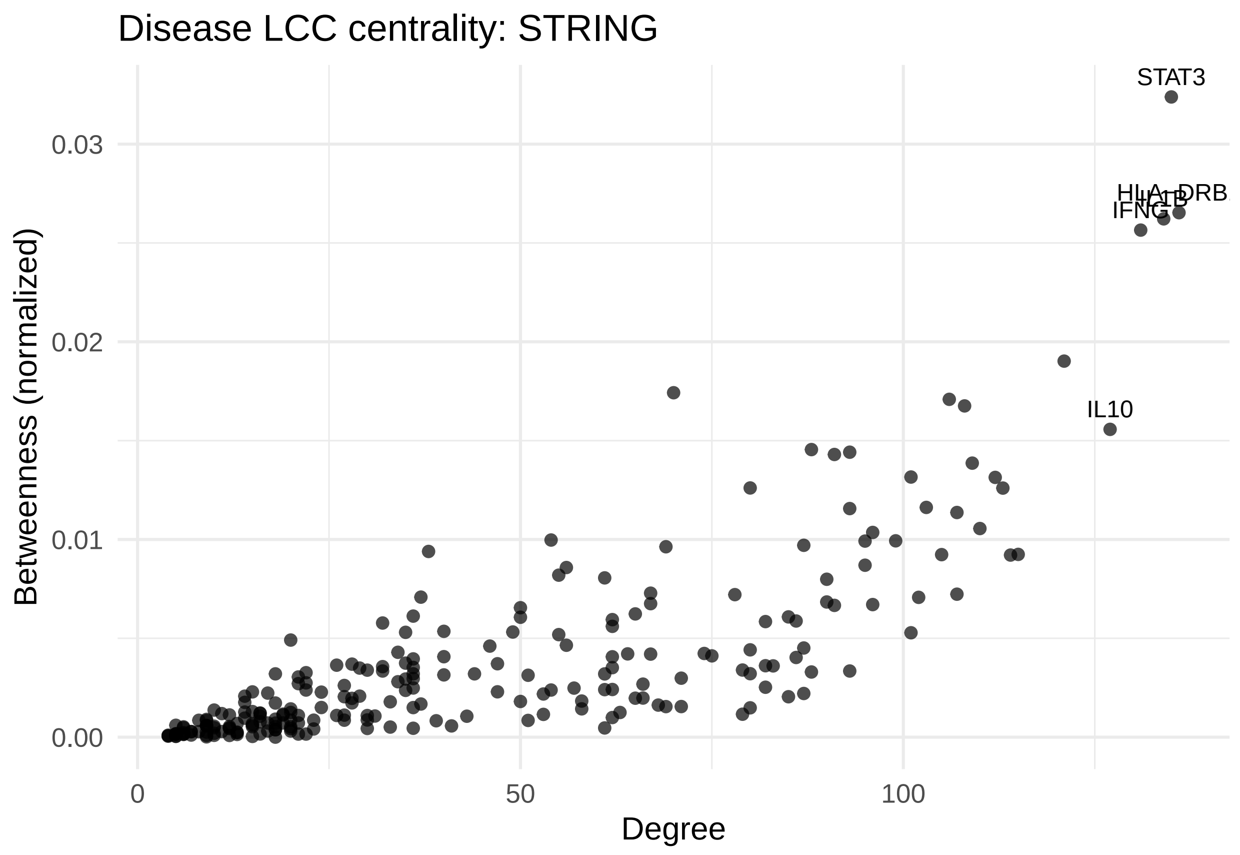
```
## # A tibble: 20 x 7
##    Interactome Gene_name Degree Betweenness Eigenvector Closeness
##    <chr>       <chr>      <dbl>       <dbl>       <dbl>     <dbl>
##  1 BioGRID     TNIP1         16      0.172       1          0.347
##  2 BioGRID     ATG16L1       15      0.162       0.515      0.322
##  3 BioGRID     HLA-A         12      0.206       0.701      0.341
##  4 BioGRID     MAPK1         10      0.0960      0.627      0.324
##  5 BioGRID     EHMT2         10      0.0918      0.150      0.277
##  6 BioGRID     CDC37          9      0.130       0.514      0.324
##  7 BioGRID     STAT3          9      0.0967      0.496      0.319
##  8 BioGRID     MCAM           8      0.122       0.311      0.305
##  9 BioGRID     TRAF3          8      0.0811      0.497      0.312
## 10 BioGRID     IQGAP1         8      0.0744      0.517      0.318
## 11 BioGRID     BAG6           8      0.0608      0.323      0.299
## 12 BioGRID     HLA-DQB1       8      0.0281      0.456      0.273
## 13 BioGRID     ERBB3          7      0.0730      0.425      0.328
## 14 BioGRID     HLA-B          7      0.0475      0.559      0.307
## 15 BioGRID     NLRP3          7      0.0369      0.203      0.265
## 16 BioGRID     HLA-DRB1       7      0.0231      0.275      0.239
## 17 BioGRID     SLC30A7        6      0.121       0.215      0.289
## 18 BioGRID     RBPJ           6      0.118       0.0586     0.259
## 19 BioGRID     ITPR3          6      0.0776      0.397      0.308
## 20 BioGRID     ICAM1          6      0.0589      0.0726     0.247
## # i 1 more variable: ratio_Betw_Degree <dbl>
```



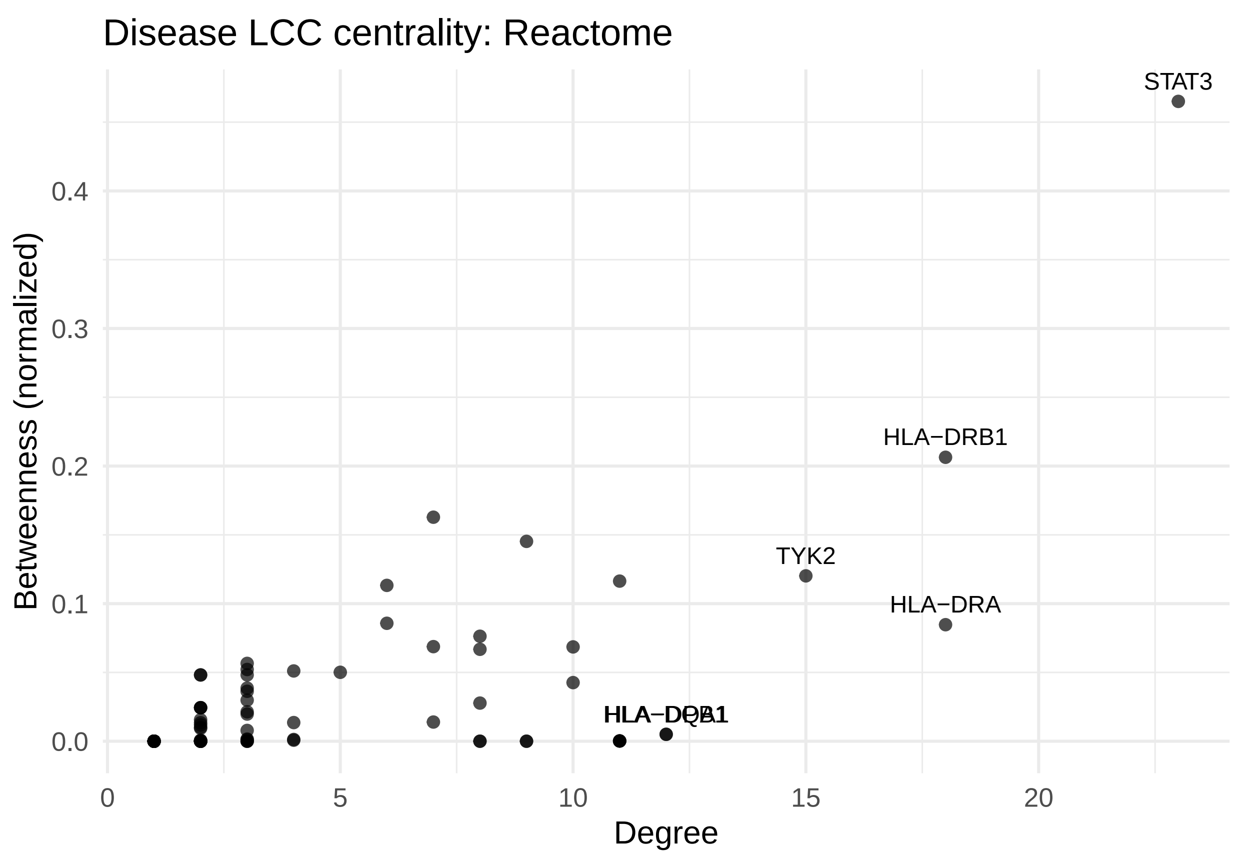
```
## # A tibble: 6 x 7
##   Interactome Gene_name Degree Betweenness Eigenvector Closeness
##   <chr>       <chr>      <dbl>       <dbl>       <dbl>     <dbl>
## 1 HuRI        CTNNA3         3         0.8       1         0.714
## 2 HuRI        CDSN           2         0.4       0.707     0.556
## 3 HuRI        ZGPAT          2         0.4       0.707     0.556
## 4 HuRI        BAG6           1         0         0.366     0.385
## 5 HuRI        TCF19          1         0         0.366     0.385
## 6 HuRI        MCCD1          1         0         0.518     0.455
## # i 1 more variable: ratio_Betw_Degree <dbl>
```



```
## # A tibble: 20 x 7
##    Interactome Gene_name Degree Betweenness Eigenvector Closeness
##    <chr>       <chr>      <dbl>       <dbl>       <dbl>     <dbl>
##  1 STRING      HLA-DRB1     136     0.0265        0.983     0.701
##  2 STRING      STAT3        135     0.0324        0.975     0.699
##  3 STRING      IL1B         134     0.0262        0.985     0.697
##  4 STRING      IFNG         131     0.0257        1         0.691
##  5 STRING      IL10         127     0.0156        0.996     0.683
##  6 STRING      PTPN22       121     0.0190        0.932     0.666
##  7 STRING      STAT4        115     0.00925       0.952     0.655
##  8 STRING      IL2RA        114     0.00921       0.948     0.655
##  9 STRING      IRF8         113     0.0126        0.921     0.657
## 10 STRING      TNFAIP3      112     0.0131        0.909     0.651
## 11 STRING      ITGAM        110     0.0106        0.932     0.648
## 12 STRING      HLA-DQB1     109     0.0139        0.882     0.648
## 13 STRING      TYK2         108     0.0168        0.857     0.644
## 14 STRING      ICAM1        107     0.0114        0.885     0.641
## 15 STRING      CD40         107     0.00723       0.928     0.641
## 16 STRING      IL7R         106     0.0171        0.905     0.642
## 17 STRING      HLA-B        105     0.00923       0.879     0.637
## 18 STRING      IFIH1        103     0.0116        0.862     0.634
## 19 STRING      IL17A        102     0.00707       0.883     0.634
## 20 STRING      VCAM1        101     0.0132        0.835     0.629
## # i 1 more variable: ratio_Betw_Degree <dbl>
```



```
## # A tibble: 20 x 7
##    Interactome Gene_name Degree Betweenness Eigenvector Closeness
##    <chr>       <chr>      <dbl>       <dbl>       <dbl>     <dbl>
##  1 Reactome    STAT3         23    0.465         0.292      0.471
##  2 Reactome    HLA-DRB1      18    0.206         1          0.412
##  3 Reactome    HLA-DRA       18    0.0847        0.984      0.363
##  4 Reactome    TYK2          15    0.120         0.369      0.427
##  5 Reactome    HLA-DPB1      12    0.00502       0.854      0.322
##  6 Reactome    HLA-DQA1      12    0.00502       0.854      0.322
##  7 Reactome    MAPK1         11    0.116         0.0870     0.392
##  8 Reactome    HLA-DQA2      11    0.000188      0.819      0.306
##  9 Reactome    HLA-DQB1      11    0.000188      0.819      0.306
## 10 Reactome    HLA-DQB2      11    0.000188      0.819      0.306
## 11 Reactome    HLA-DRB4      11    0.000188      0.819      0.306
## 12 Reactome    IL12A         10    0.0686        0.340      0.416
## 13 Reactome    STAT4         10    0.0426        0.356      0.392
## 14 Reactome    IL2RB          9    0.145         0.145      0.385
## 15 Reactome    HLA-DMA        9    0             0.710      0.304
## 16 Reactome    HLA-DMB        9    0             0.710      0.304
## 17 Reactome    HLA-A          8    0.0763        0.139      0.340
## 18 Reactome    CD86           8    0.0668        0.409      0.349
## 19 Reactome    IL2RA          8    0.0277        0.198      0.387
## 20 Reactome    PDCD1          8    0             0.645      0.303
## # i 1 more variable: ratio_Betw_Degree <dbl>
```



```r
message("Table 2 completed for all interactomes.")
```

Save Part 1 Objects

```r
saveRDS(
  list(
    interactomes_lcc = interactomes_lcc,
    seed_genes = seed_genes,
    table1 = table1
  ),
  file.path(OUTDIR, "PART1_objects.rds")
)

message("Part 1 complete. Outputs saved to: ", OUTDIR)
```

Part 2: Gene Prioritization

Configuration

```r
set.seed(123)
N_FOLDS <- 5
TIMES <- c(0.1, 1.0, 2.0, 5.0, 10.0)
DIFF_ITERS <- 20
```

```r
python_exe <- "C:/Users/Utente/AppData/Local/Programs/Python/Python314/python.exe"

dir.create("PART2_outputs", showWarnings = FALSE, recursive = TRUE)
dir.create("PART2_outputs/cv_diamond", showWarnings = FALSE, recursive = TRUE)
dir.create("PART2_outputs/cv_diffusion", showWarnings = FALSE, recursive = TRUE)

stopifnot(file.exists("DIAMOnD.py"))
stopifnot(file.exists(python_exe))
```

Performance Metrics

```r
score_ranking <- function(ranked_genes, probe_set, K) {
  ranked_genes <- ranked_genes[!is.na(ranked_genes) & ranked_genes != ""]
  K_eff <- min(K, length(ranked_genes))
  if (K_eff <= 0) return(tibble(Precision = NA_real_, Recall = NA_real_, F1 = NA_real_, Hits = 0L, K_ef

  topK <- ranked_genes[seq_len(K_eff)]
  hits <- sum(topK %in% probe_set)

  precision <- hits / K_eff
  recall <- if (length(probe_set) == 0) 0 else hits / length(probe_set)
  f1 <- if ((precision + recall) == 0) 0 else 2 * precision * recall / (precision + recall)

  tibble(Precision = precision, Recall = recall, F1 = f1, Hits = hits, K_eff = K_eff)
}

make_K_values <- function(n) {
K <- unique(round(c(50, n/10, n/4, n/2, n)))
sort(K[K > 0])
}
```

Diffusion Functions

```r
## Diffusion Functions (HEAT DIFFUSION / NETWORK PROPAGATION)

# We implement heat diffusion using the random-walk normalized Laplacian:
#   L_rw = I - D^{-1} A  where W = D^{-1}A is the row-stochastic walk matrix.
# Heat diffusion solves:  dp/dt = - L_rw p
# Discretized (explicit Euler): p_{k+1} = p_k - dt * L_rw p_k
#                              = (1 - dt) p_k + dt * W p_k
#
# Here, the diffusion "time" t controls how far we propagate:
# total simulated time = n_steps * dt   t

prepare_diffusion <- function(g) {
  A <- igraph::as_adjacency_matrix(g, sparse = TRUE)
  d <- igraph::degree(g)
  d_inv <- ifelse(d > 0, 1 / d, 0)
  W <- Matrix::Diagonal(x = d_inv) %*% A  # row-stochastic random-walk matrix
  list(W = W, nodes = igraph::V(g)$name)
}
```

```r
run_diffusion <- function(prep, seeds, t, dt = 0.01) {
  nodes <- prep$nodes
  W <- prep$W
  n <- length(nodes)

  # seeds restricted to graph
  seeds <- intersect(seeds, nodes)

  # initial heat: 1 on seeds, 0 elsewhere; normalize to sum to 1 (probability-like)
  p <- numeric(n); names(p) <- nodes
  p[seeds] <- 1
  if (sum(p) > 0) p <- p / sum(p)

  # number of Euler steps so that total diffusion time approx equals t
  t <- as.numeric(t)
  if (is.na(t) || t < 0) stop("t must be a non-negative number")
  dt <- as.numeric(dt)
  if (is.na(dt) || dt <= 0 || dt > 1) stop("dt must be in (0, 1] for stability")

  n_steps <- max(1L, as.integer(ceiling(t / dt)))

  # Heat diffusion iterations (no restart term)
  p_mat <- as.matrix(p)
  for (k in seq_len(n_steps)) {
    p_mat <- (1 - dt) * p_mat + dt * (W %*% p_mat)
  }

  scores <- as.vector(p_mat); names(scores) <- nodes

  # IMPORTANT (project spec): seed genes appear mixed in the ranking; remove them before evaluation :co
  scores[seeds] <- -Inf

  sort(scores, decreasing = TRUE)
}
```

DIAMOnD Functions

```r
run_diamond <- function(netfile, seeds, max_needed, outpath, python_exe) {
  write.table(seeds, "current_seeds.txt", row.names = FALSE, col.names = FALSE, quote = FALSE)

  cmd <- paste(
    shQuote(python_exe),
    shQuote("DIAMOnD.py"),
    shQuote(netfile),
    shQuote("current_seeds.txt"),
    as.character(max_needed),
    "1",
    shQuote(outpath)
  )

  system(cmd, intern = FALSE, ignore.stdout = TRUE, ignore.stderr = FALSE)

  if (!file.exists(outpath)) {
    stop("DIAMOnD failed: output not created at ", outpath)
  }
}

read_diamond_rank <- function(path) {
  lines <- readLines(path, warn = FALSE)
  lines <- lines[!grepl("^\\s*#", lines)]
  lines <- lines[nzchar(lines)]
  parts <- strsplit(lines, "\\s+")
  genes <- vapply(parts, function(x) if (length(x) >= 2) x[[2]] else NA_character_, character(1))
  genes <- genes[!is.na(genes) & genes != ""]
  genes
}
```

Cross-Validation Loop

```r
# NOTA: Questo chunk è impostato con eval=FALSE perché richiede molto tempo.
# I risultati sono già salvati in PART2_outputs/cv_all_folds_results.csv
all_rows <- list()
row_i <- 1

for (net_name in names(interactomes_lcc)) {

  message("Processing: ", net_name)

  g <- interactomes_lcc[[net_name]]
  universe <- V(g)$name

  seeds_present <- intersect(seed_genes, universe)
  n <- length(seeds_present)

  if (n < 10) {
    warning("Too few disease genes in LCC for ", net_name)
    next
  }

  K_values = make_K_values(n)

  # DIAMOnD must output at least n candidates (module expansion length)
  diamond_iters_needed <- max(K_values)


  prep <- prepare_diffusion(g)

  shuffled <- sample(seeds_present)
  fold_id <- cut(seq_along(shuffled), breaks = N_FOLDS, labels = FALSE)

  netfile <- paste0(net_name, "_network.txt")
  if (!file.exists(netfile)) stop("Missing DIAMOnD network file: ", netfile)

  for (f in seq_len(N_FOLDS)) {

    probe <- shuffled[fold_id == f]
    train <- shuffled[fold_id != f]

    # DIAMOnD
    diamond_out <- file.path("PART2_outputs/cv_diamond", paste0("diamond_", net_name, "_fold", f, ".txt

    if (!file.exists(diamond_out)) {
      run_diamond(netfile, train, diamond_iters_needed, diamond_out, python_exe)
    }

    diamond_rank <- read_diamond_rank(diamond_out)
    diamond_rank <- setdiff(diamond_rank, train)

    if (length(diamond_rank) < max(K_values)) {
      warning("DIAMOnD produced only ", length(diamond_rank),
              " candidates but max(K)=", max(K_values),
              " for net=", net_name, " fold=", f,
              ". Consider increasing diamond_iters_needed.")
    }

    for (K in K_values) {
      perf <- score_ranking(diamond_rank, probe, K)
      all_rows[[row_i]] <- tibble(
        Network = net_name,
        Algo = "DIAMOnD",
        Parameter = NA_real_,
        Fold = f,
        TopK = K,
        Precision = perf$Precision,
        Recall = perf$Recall,
        F1 = perf$F1,
        Hits = perf$Hits
      )
      row_i <- row_i + 1
}

    # Diffusion
    for (t_val in TIMES) {

      diff_scores <- run_diffusion(prep, train, t = t_val, dt = 0.01)
      diff_rank <- names(diff_scores)
      diff_rank <- diff_rank[!(diff_rank %in% train)]
      t_tag <- gsub("\\.", "p", as.character(t_val))
      diff_out <- file.path("PART2_outputs/cv_diffusion", paste0("diff_", net_name, "_fold", f, "_t", t
      if (!file.exists(diff_out)) {
        write_csv(tibble(Gene = diff_rank, Score = as.numeric(diff_scores[diff_rank])), diff_out)
      }

      for (K in K_values) {
        perf <- score_ranking(diff_rank, probe, K)
        all_rows[[row_i]] <- tibble(
          Network = net_name,
          Algo = "Diffusion",
          Parameter = t_val,
          Fold = f,
          TopK = K,
          Precision = perf$Precision,
          Recall = perf$Recall,
          F1 = perf$F1,
          Hits = perf$Hits
        )

        row_i <- row_i + 1
      }
    }
  }
}

results_all <- bind_rows(all_rows)
```

Save Results

```
# Carica i risultati già calcolati invece di ricalcolarli
if (!exists("results_all")) {
  results_all <- read_csv("PART2_outputs/cv_all_folds_results.csv", show_col_types = FALSE)
}

write_csv(results_all, "PART2_outputs/cv_all_folds_results.csv")

summary_results <- results_all %>%
  group_by(Network, Algo, Parameter, TopK) %>%
  summarise(
    Precision_mean = mean(Precision, na.rm = TRUE),
    Precision_sd   = sd(Precision, na.rm = TRUE),
    Recall_mean    = mean(Recall, na.rm = TRUE),
    Recall_sd      = sd(Recall, na.rm = TRUE),
    F1_mean        = mean(F1, na.rm = TRUE),
    F1_sd          = sd(F1, na.rm = TRUE),
    Hits_mean      = mean(Hits, na.rm = TRUE),
    .groups = "drop"
  )

write_csv(summary_results, "PART2_outputs/cv_summary_mean_sd.csv")
```

```
# --- Always define best_network/best_algo/best_param (even if CV chunk is eval=FALSE) ---

# 1) Make sure summary_results exists (load from disk if needed)
if (!exists("summary_results")) {
  if (file.exists("PART2_outputs/cv_summary_mean_sd.csv")) {
    summary_results <- readr::read_csv("PART2_outputs/cv_summary_mean_sd.csv", show_col_types = FALSE)
  } else {
    stop("Missing summary_results and PART2_outputs/cv_summary_mean_sd.csv. Run CV once or provide the 
  }
}

# 2) Pick TopK=50 if available, otherwise use the largest TopK present
target_topk <- 24
```

```r
if (!(target_topk %in% summary_results$TopK)) {
  target_topk <- max(summary_results$TopK, na.rm = TRUE)
  message("TopK=50 not available; using TopK=", target_topk)
}

# 3) Compute the best combination
best_combo <- summary_results %>%
  dplyr::filter(TopK == target_topk) %>%
  dplyr::arrange(
    dplyr::desc(F1_mean),
    dplyr::desc(Precision_mean),
    dplyr::desc(Recall_mean),
    F1_sd
  ) %>%
  dplyr::slice(1)


best_network <- best_combo$Network[[1]]
best_algo    <- best_combo$Algo[[1]]
best_param   <- best_combo$Parameter[[1]]

message("Best combo @TopK=", target_topk, ": ", best_algo, " on ", best_network,
        ifelse(is.na(best_param), "", paste0(" (t=", best_param, ")")))
```

Identify Best Performer

```r
print(best_combo)
```

```
## # A tibble: 1 x 11
##   Network Algo    Parameter  TopK Precision_mean Precision_sd Recall_mean
##   <chr>   <chr>       <dbl> <dbl>          <dbl>        <dbl>       <dbl>
## 1 STRING  DIAMOnD        NA    24          0.217       0.0186       0.109
## # i 4 more variables: Recall_sd <dbl>, F1_mean <dbl>, F1_sd <dbl>,
## #   Hits_mean <dbl>
```

Visualizations

```r
dir.create("PART2_outputs/figures", showWarnings = FALSE, recursive = TRUE)

p1 <- ggplot(summary_results %>% filter(TopK == 50)
, aes(x = Network, y = Precision_mean, fill = Algo)) +
  geom_col(position = position_dodge(width = 0.8)) +
  geom_errorbar(aes(ymin = Precision_mean - Precision_sd,
                    ymax = Precision_mean + Precision_sd),
                width = 0.2, position = position_dodge(width = 0.8)) +
  facet_wrap(~ Parameter, labeller = label_both) +
  theme_minimal(base_size = 13) +
  labs(title = "Top-50 Precision across interactomes",
       subtitle = "Mean ± SD over 5-fold CV",
       y = "Precision", x = "Interactome")

ggsave("PART2_outputs/figures/PART2_precision_top50_bar.png", p1, width = 12, height = 6, dpi = 300)
print(p1)
```

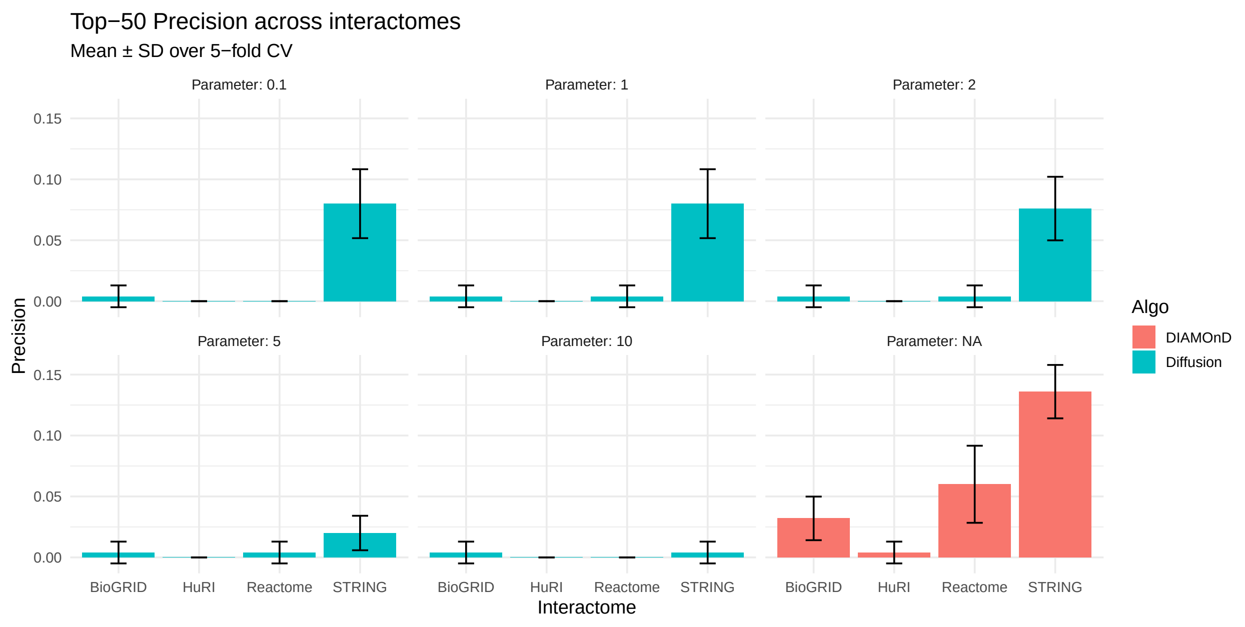


Figure 1: Top-50 Precision across interactomes (Mean ± SD over 5-fold CV)

```
p2 <- ggplot(summary_results %>% filter(TopK == 50),
             aes(x = Recall_mean, y = Precision_mean, color = Algo, shape = Network)) +
  geom_point(size = 3) +
  facet_wrap(~ Parameter) +
  theme_minimal(base_size = 13) +
  labs(title = "Precision-Recall tradeoff (Top-50)",
       x = "Recall", y = "Precision")

ggsave("PART2_outputs/figures/PART2_precision_recall_top50.png", p2, width = 12, height = 6, dpi = 300)
print(p2)
```

```
p3 <- ggplot(summary_results,
             aes(x = factor(TopK), y = F1_mean,
                 group = interaction(Algo, Parameter),
                 color = Algo)) +
  geom_line() + geom_point() +
  facet_grid(Network ~ Parameter) +
  theme_minimal(base_size = 12) +
  labs(title = "F1-score across Top-K thresholds",
       x = "Top-K", y = "Mean F1")

ggsave("PART2_outputs/figures/PART2_f1_across_topk.png", p3, width = 12, height = 10, dpi = 300)
print(p3)
```

```
# Ensure viridis is available
if (!requireNamespace("viridis", quietly = TRUE)) install.packages("viridis")
library(viridis)

# Build heatmap data at TopK = 50
heat_data <- summary_results %>%
```

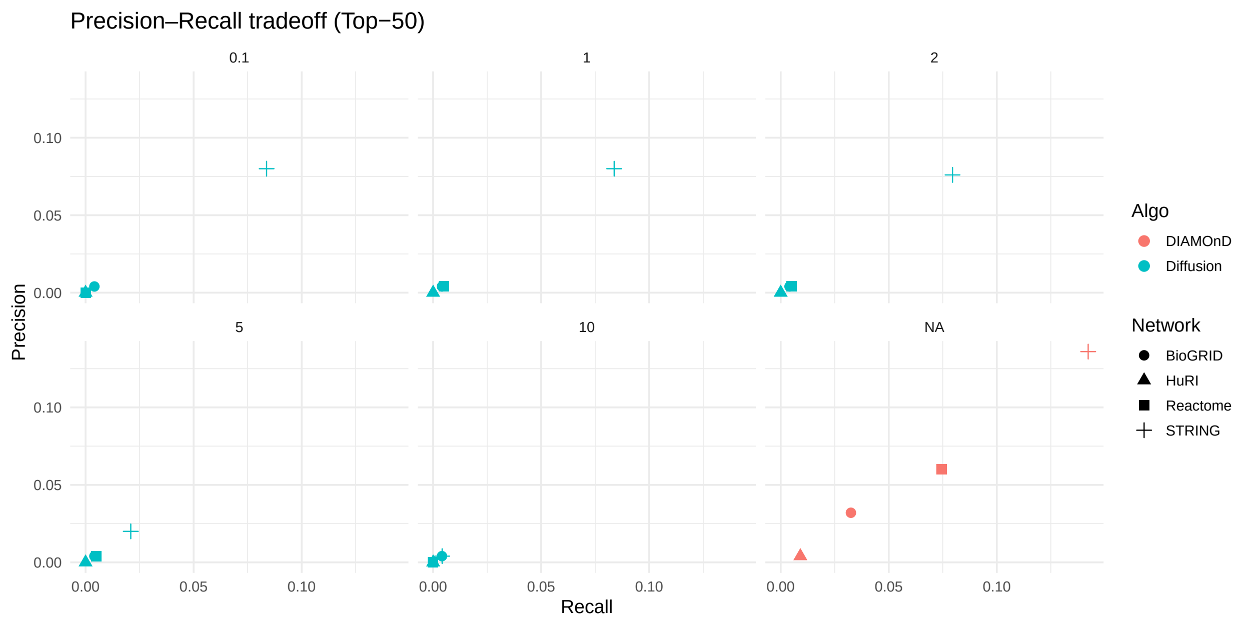


Figure 2: Precision-Recall tradeoff at Top-50

```
  filter(TopK == 50) %>%
  mutate(
    Method = ifelse(is.na(Parameter),
                    Algo,
                    paste0(Algo, "_t=", Parameter))
  ) %>%
  dplyr::select(Network, Method, Precision_mean)

# Plot
p_heat <- ggplot(heat_data, aes(x = Method, y = Network, fill = Precision_mean)) +
  geom_tile(color = "white") +
  scale_fill_viridis_c(option = "C") +
  theme_minimal(base_size = 12) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
  labs(
    title = "Precision heatmap (Top-50)",
    x = "Method",
    y = "Interactome",
    fill = "Mean Precision"
  )

ggsave("PART2_outputs/figures/PART2_precision_top50_heatmap.png",
       p_heat, width = 12, height = 5.5, dpi = 300)

print(p_heat)
```

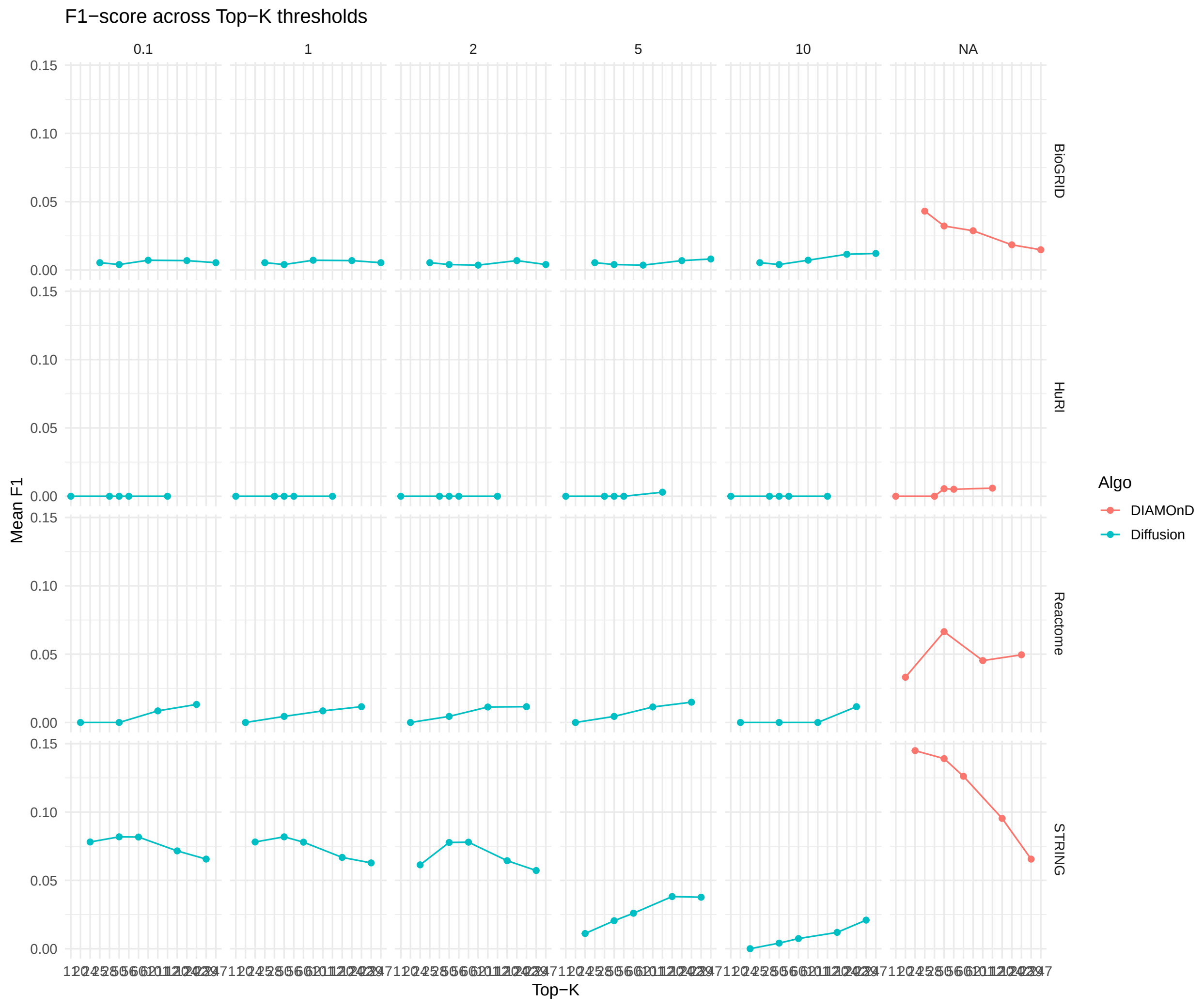


Figure 3: F1-score across Top-K thresholds

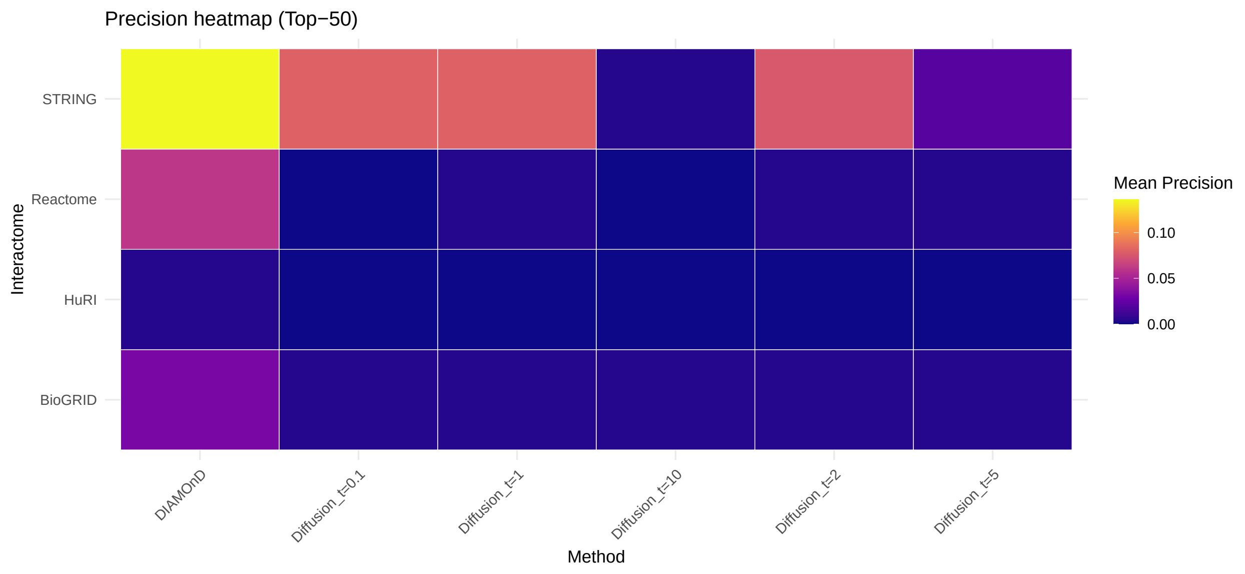


Figure 4: Precision heatmap (Top-50) across interactomes and methods

Part 3: Enrichment Analysis

Setup

```r
dir.create("PART3_outputs", showWarnings = FALSE, recursive = TRUE)
dir.create("PART3_outputs/final_run", showWarnings = FALSE, recursive = TRUE)
dir.create("PART3_outputs/enrichment", showWarnings = FALSE, recursive = TRUE)
dir.create("PART3_outputs/figures", showWarnings = FALSE, recursive = TRUE)
```

Generate Putative Genes

```r
TOP_N <- 100
DIFF_ITERS <- 20

g_best <- interactomes_lcc[[best_network]]
universe <- V(g_best)$name

final_seeds <- intersect(seed_genes, universe)
writeLines(final_seeds, "PART3_outputs/final_run/final_seeds_in_best_interactome.txt")

message("Universe size: ", length(universe))
message("Seeds in universe: ", length(final_seeds))
```

```r
# NOTA: Questo chunk è impostato con eval=FALSE perché DIAMOnD/Diffusion richiedono tempo.
# I risultati sono già salvati in PART3_outputs/final_run/putative_genes_top100.csv
best_algo_norm <- tolower(trimws(best_algo))

if (best_algo_norm == "diamond") {

  netfile <- paste0(best_network, "_network.txt")
  diamond_out <- "PART3_outputs/final_run/diamond_final_results.txt"
  run_diamond(netfile, final_seeds, TOP_N, diamond_out, python_exe)
  diamond_rank <- read_diamond_rank(diamond_out)
  diamond_rank <- setdiff(diamond_rank, final_seeds)

  putative_genes <- diamond_rank[seq_len(TOP_N)]
  putative_tbl <- tibble(
    Rank = seq_len(TOP_N),
    Gene = putative_genes,
    Score = NA_real_,
    Method = "DIAMOnD",
    Network = best_network,
    Parameter = NA_real_
  )

} else if (best_algo_norm == "diffusion") {

  prep <- prepare_diffusion(g_best)
  scores <- run_diffusion(prep, final_seeds, t = as.numeric(best_param), dt = 0.01)

  ranked_genes <- names(scores)
  ranked_genes <- ranked_genes[!(ranked_genes %in% final_seeds)]

  putative_genes <- ranked_genes[seq_len(TOP_N)]
  putative_tbl <- tibble(
    Rank = seq_len(TOP_N),
    Gene = putative_genes,
    Score = as.numeric(scores[putative_genes]),
    Method = "Diffusion",
    Network = best_network,
    Parameter = as.numeric(best_param)
  )
}

write_csv(putative_tbl, "PART3_outputs/final_run/putative_genes_top100.csv")
writeLines(putative_genes, "PART3_outputs/final_run/putative_genes_top100.txt")

print(head(putative_tbl, 15))
```

```r
# Carica i risultati già calcolati
if (!exists("putative_tbl")) {
  putative_tbl <- read_csv("PART3_outputs/final_run/putative_genes_top100.csv", show_col_types = FALSE)
  putative_genes <- putative_tbl$Gene
}

print(head(putative_tbl, 20))
```

```
## # A tibble: 20 x 6
##     Rank Gene     Score Method  Network Parameter
##    <dbl> <chr>    <lgl> <chr>   <chr>   <lgl>
##  1     1 TNFRSF25 NA    DIAMOnD STRING  NA
##  2     2 IRF5     NA    DIAMOnD STRING  NA
##  3     3 IL18R1   NA    DIAMOnD STRING  NA
##  4     4 SH2B3    NA    DIAMOnD STRING  NA
##  5     5 BLK      NA    DIAMOnD STRING  NA
## 6      6 CTLA4    NA    DIAMOnD STRING  NA
## 7      7 HLA-C    NA    DIAMOnD STRING  NA
## 8      8 IL18RAP  NA    DIAMOnD STRING  NA
## 9      9 CCRL2    NA    DIAMOnD STRING  NA
## 10    10 CIITA    NA    DIAMOnD STRING  NA
## 11    11 TNFSF4   NA    DIAMOnD STRING  NA
## 12    12 TBX21    NA    DIAMOnD STRING  NA
## 13    13 TNFRSF14 NA    DIAMOnD STRING  NA
## 14    14 CD28     NA    DIAMOnD STRING  NA
## 15    15 IL21R    NA    DIAMOnD STRING  NA
## 16    16 FCGR2A   NA    DIAMOnD STRING  NA
## 17    17 BTLA     NA    DIAMOnD STRING  NA
## 18    18 TNFRSF4  NA    DIAMOnD STRING  NA
## 19    19 CD247    NA    DIAMOnD STRING  NA
## 20    20 CD83     NA    DIAMOnD STRING  NA
```

EnrichR Analysis

```r
if (!requireNamespace("enrichR", quietly = TRUE)) install.packages("enrichR")
library(enrichR)

ENRICHR_LIBS <- c(
  "GO_Biological_Process_2021",
  "GO_Molecular_Function_2021",
  "GO_Cellular_Component_2021",
  "KEGG_2021_Human",
  "Reactome_2022"
)

PADJ_CUTOFF <- 0.05

clean_gene_list <- function(x) {
  x <- as.character(x)
  x <- x[!is.na(x)]
  x <- trimws(x)
  x <- x[x != ""]
  unique(x)
}

seeds_for_enrichment <- clean_gene_list(final_seeds)
putative_for_enrichment <- clean_gene_list(putative_genes)

message("Enrichment seeds (n=", length(seeds_for_enrichment), ") | putative (n=", length(putative_for_e
```

```r
enr_put  <- enrichr(putative_for_enrichment, ENRICHR_LIBS)
```

```
## Uploading data to Enrichr... Done.
##   Querying GO_Biological_Process_2021... Done.
##   Querying GO_Molecular_Function_2021... Done.
##   Querying GO_Cellular_Component_2021... Done.
##   Querying KEGG_2021_Human... Done.
##   Querying Reactome_2022... Done.
## Parsing results... Done.
```

```
enr_seed <- enrichr(seeds_for_enrichment,   ENRICHR_LIBS)
```

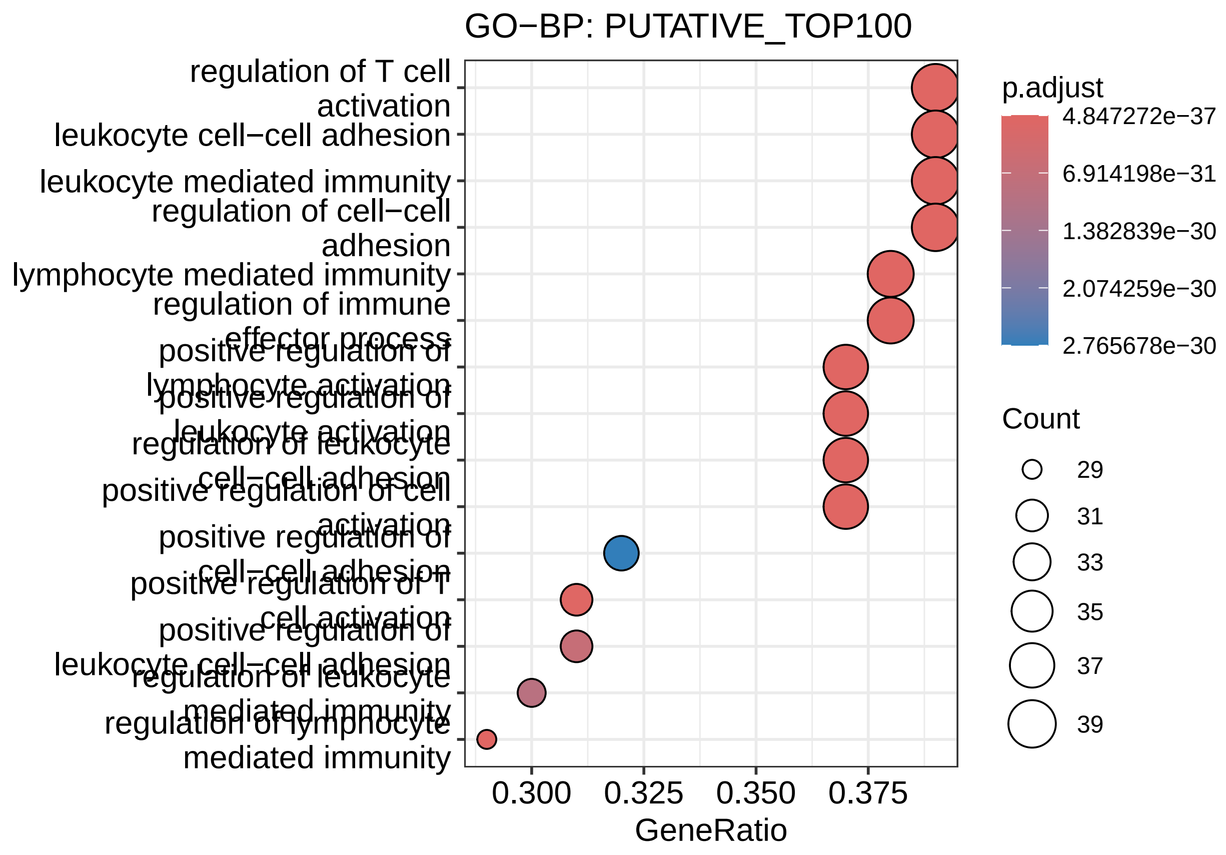
```
## Uploading data to Enrichr... Done.
##   Querying GO_Biological_Process_2021... Done.
##   Querying GO_Molecular_Function_2021... Done.
##   Querying GO_Cellular_Component_2021... Done.
##   Querying KEGG_2021_Human... Done.
##   Querying Reactome_2022... Done.
## Parsing results... Done.
```

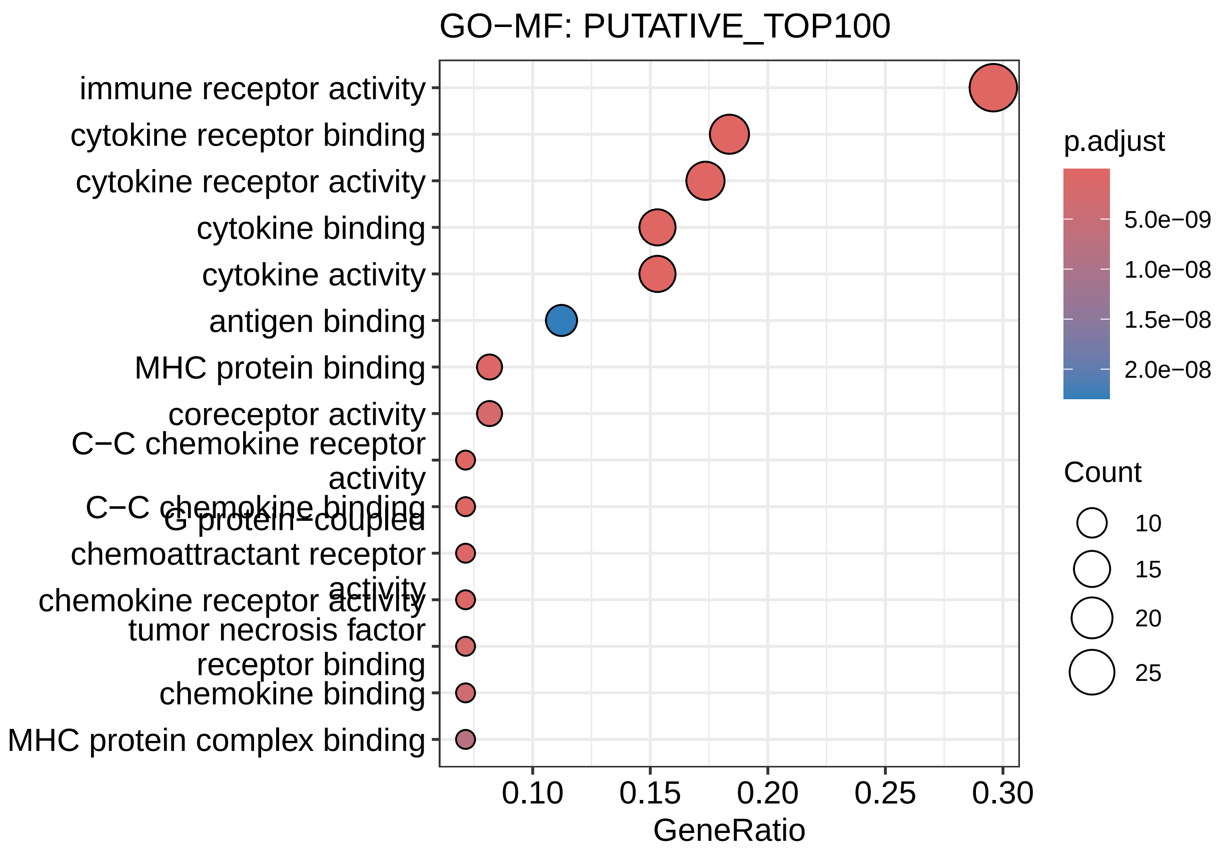
```
standardize_enrichr <- function(df, db_name) {
  if (is.null(df) || nrow(df) == 0) return(tibble::tibble())

  df <- tibble::as_tibble(df)
  df <- dplyr::mutate(df, Database = db_name)

  for (nm in c("Adjusted.P.value", "P.value", "Combined.Score", "Odds.Ratio")) {
    if (nm %in% names(df)) {
      df[[nm]] <- suppressWarnings(as.numeric(df[[nm]]))
    }
  }

  if ("Overlap" %in% names(df)) {
    df <- dplyr::mutate(
      df,
      Overlap = as.character(.data$Overlap),
      Overlap_k = suppressWarnings(as.integer(sub("/.*", "", .data$Overlap))),
      Overlap_n = suppressWarnings(as.integer(sub(".*/", "", .data$Overlap)))
    )
  }

  if ("Genes" %in% names(df)) df$Genes <- as.character(df$Genes)
  if ("Term"  %in% names(df)) df$Term  <- as.character(df$Term)

  preferred <- c(
    "Database", "Term", "Adjusted.P.value", "P.value",
    "Overlap", "Overlap_k", "Overlap_n",
    "Odds.Ratio", "Combined.Score", "Genes"
  )
  keep_front <- preferred[preferred %in% names(df)]

  dplyr::select(df, dplyr::all_of(keep_front), dplyr::everything())
}

save_enrichr_outputs <- function(enr_list, label, padj = 0.05, top_show = 20) {
  all_tbls <- list()
  sig_tbls <- list()
  top_tbls <- list()

  for (db in names(enr_list)) {
    df <- standardize_enrichr(enr_list[[db]], db)

    out_all <- file.path("PART3_outputs/enrichment", paste0(label, "_", db, "_ALL.csv"))
    readr::write_csv(df, out_all)
    all_tbls[[db]] <- df

    df_sig <- if ("Adjusted.P.value" %in% names(df)) {
      dplyr::filter(df, !is.na(.data$Adjusted.P.value) & .data$Adjusted.P.value < padj)
    } else {
      tibble::tibble()
    }
    out_sig <- file.path("PART3_outputs/enrichment", paste0(label, "_", db, "_SIG_padj", padj, ".csv"))
    readr::write_csv(df_sig, out_sig)
    sig_tbls[[db]] <- df_sig

    df_top <- if (nrow(df_sig) > 0) {
      if ("Combined.Score" %in% names(df_sig)) {
        dplyr::arrange(df_sig, .data$Adjusted.P.value, dplyr::desc(.data$Combined.Score)) %>%
          dplyr::slice_head(n = top_show)
      } else {
        dplyr::arrange(df_sig, .data$Adjusted.P.value) %>%
          dplyr::slice_head(n = top_show)
      }
    } else {
      tibble::tibble()
    }

    out_top <- file.path("PART3_outputs/enrichment", paste0(label, "_", db, "_TOP", top_show, "_SIG.csv
    readr::write_csv(df_top, out_top)
    top_tbls[[db]] <- df_top
  }

  list(all = all_tbls, sig = sig_tbls, top = top_tbls)
}

out_put  <- save_enrichr_outputs(enr_put,  "PUTATIVE", padj = PADJ_CUTOFF, top_show = 20)
out_seed <- save_enrichr_outputs(enr_seed, "SEEDS",    padj = PADJ_CUTOFF, top_show = 20)
```

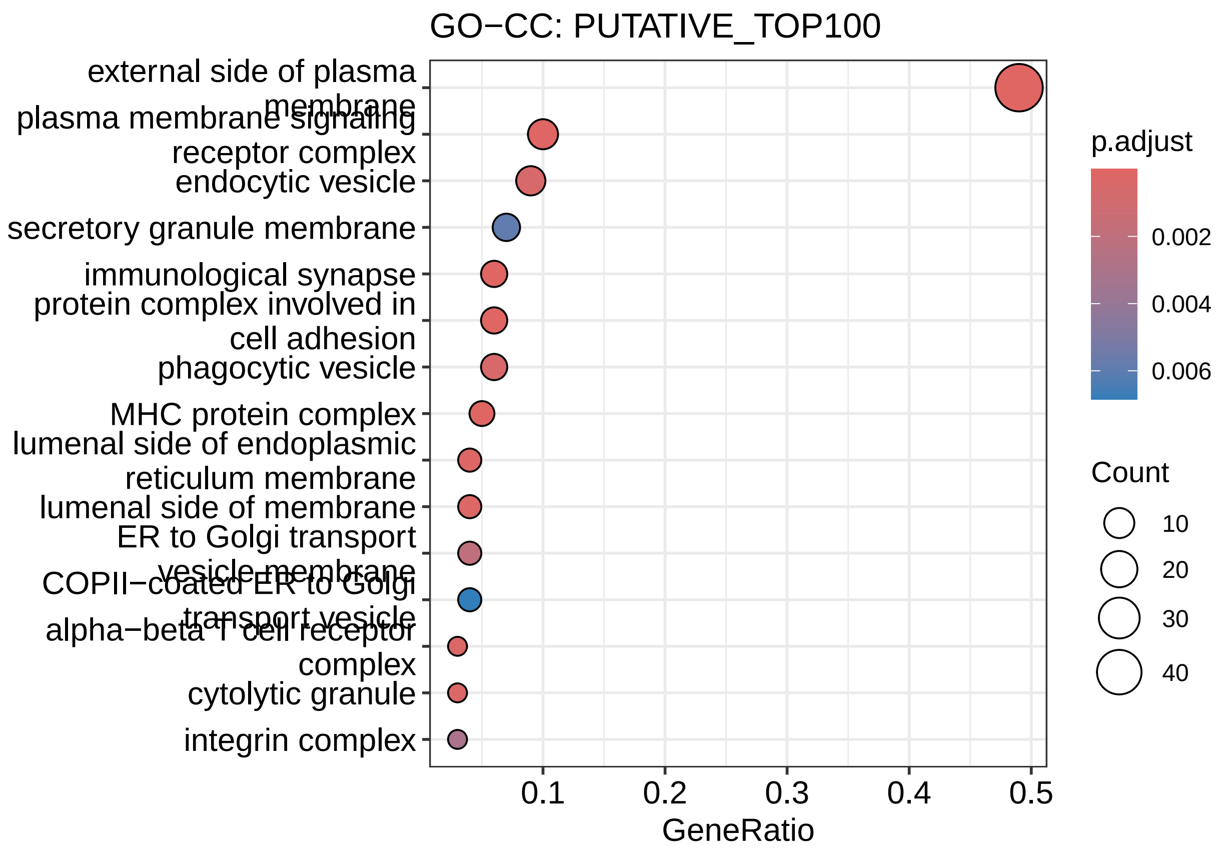
```
normalize_term <- function(term_vec, db) {
  x <- as.character(term_vec)
  x <- trimws(x)
  x <- x[x != "" & !is.na(x)]

  if (grepl("^Reactome", db)) {
    x <- sub("\\s*\\(R-HSA-[0-9]+\\)\\s*$", "", x)
  }
  if (grepl("^KEGG", db)) {
    x <- sub("\\s*-\\s*Homo\\s+sapiens\\s*\\(human\\)\\s*$", "", x)
  }
  unique(x)
}

compute_overlap <- function(sig_put, sig_seed, libs, padj = 0.05) {
  rows <- list()

  for (db in libs) {
    put_df  <- sig_put[[db]]
    seed_df <- sig_seed[[db]]
    put_terms_raw  <- if (!is.null(put_df)  && nrow(put_df)  > 0 && "Term" %in% names(put_df))  put_df$
    seed_terms_raw <- if (!is.null(seed_df) && nrow(seed_df) > 0 && "Term" %in% names(seed_df)) seed_df

    put_terms  <- normalize_term(put_terms_raw,  db)
    seed_terms <- normalize_term(seed_terms_raw, db)

    overlap_terms <- intersect(put_terms, seed_terms)
    union_terms <- union(put_terms, seed_terms)
    jaccard <- if (length(union_terms) == 0) NA_real_ else length(overlap_terms) / length(union_terms)

    writeLines(overlap_terms, file.path("PART3_outputs/enrichment", paste0("OVERLAP_terms_", db, "_padj

    rows[[db]] <- tibble(
      Database = db,
      Putative_sig_terms = length(put_terms),
      Seeds_sig_terms    = length(seed_terms),
      Overlap_terms      = length(overlap_terms),
      Jaccard            = jaccard
    )
  }

  bind_rows(rows)
}

overlap_summary <- compute_overlap(out_put$sig, out_seed$sig, ENRICHR_LIBS, padj = PADJ_CUTOFF)
write_csv(overlap_summary, "PART3_outputs/enrichment/OVERLAP_summary.csv")

print(overlap_summary)
```

```
## # A tibble: 5 x 5
##   Database              Putative_sig_terms Seeds_sig_terms Overlap_terms Jaccard
##   <chr>                              <int>           <int>         <int>   <dbl>
## 1 GO_Biological_Proces~                386             352           147  0.249
## 2 GO_Molecular_Functio~                 28               6             3  0.0968
## 3 GO_Cellular_Componen~                 28              36            16  0.333
## 4 KEGG_2021_Human                       46              71            45  0.625
## 5 Reactome_2022                         62              52            26  0.295
```

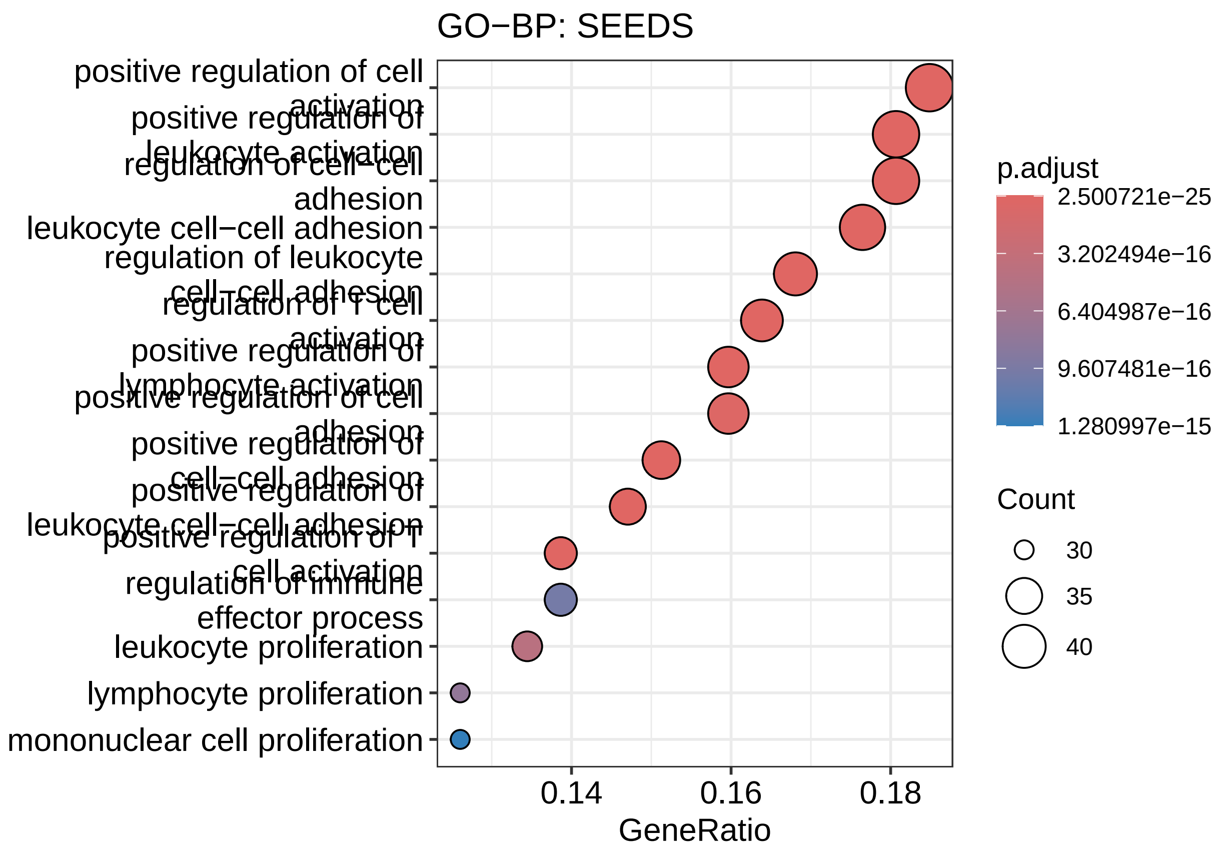
```
# ----------------------------------------------------------------------
#  clusterProfiler / ReactomePA enrichment dotplots
# ----------------------------------------------------------------------

# Directory for figures (already created above, but safe)
dir.create("PART3_outputs/figures", showWarnings = FALSE, recursive = TRUE)

# Helper: SYMBOL -> ENTREZID robust conversion
symbol_to_entrez <- function(symbols) {
  symbols <- unique(trimws(as.character(symbols)))
  symbols <- symbols[!is.na(symbols) & symbols != ""]
  conv <- suppressMessages(
    clusterProfiler::bitr(
      symbols,
      fromType = "SYMBOL",
      toType   = "ENTREZID",
      OrgDb    = org.Hs.eg.db
    )
  )
  unique(conv$ENTREZID)
}

# Prepare Entrez IDs for both sets
entrez_putative <- symbol_to_entrez(putative_genes)
entrez_seeds    <- symbol_to_entrez(seed_genes)

message("clusterProfiler IDs: putative Entrez n=", length(entrez_putative),
        " | seeds Entrez n=", length(entrez_seeds))

# Safety: stop early if conversion fails
if (length(entrez_putative) < 5 || length(entrez_seeds) < 5) {
  warning("Too few Entrez IDs after conversion. Dotplots may be uninformative. Check gene symbols / mapp
}

# ---------- GO dotplots (BP / MF / CC) ----------
run_go_dotplots <- function(entrez_vec, label, showCategory = 15, pcut = 0.05) {

  for (ont in c("BP", "MF", "CC")) {
    ego <- suppressMessages(
      clusterProfiler::enrichGO(
        gene          = entrez_vec,
        OrgDb         = org.Hs.eg.db,
        ont           = ont,
        pvalueCutoff  = pcut,
        readable      = TRUE
      )
    )

    # Print table preview in the report
    message("GO-", ont, " terms for ", label, ": ", nrow(as.data.frame(ego)))

    p <- enrichplot::dotplot(ego, showCategory = showCategory) +
      ggplot2::ggtitle(paste0("GO-", ont, ": ", label))

    out_png <- file.path("PART3_outputs/figures", paste0("PART3_dotplot_GO_", ont, "_", label, ".png"))
    ggsave(out_png, p, width = 10, height = 6, dpi = 300)
    print(p)
  }
}

# Putative GO
run_go_dotplots(entrez_putative, label = "PUTATIVE_TOP100", showCategory = 15, pcut = 0.05)
```

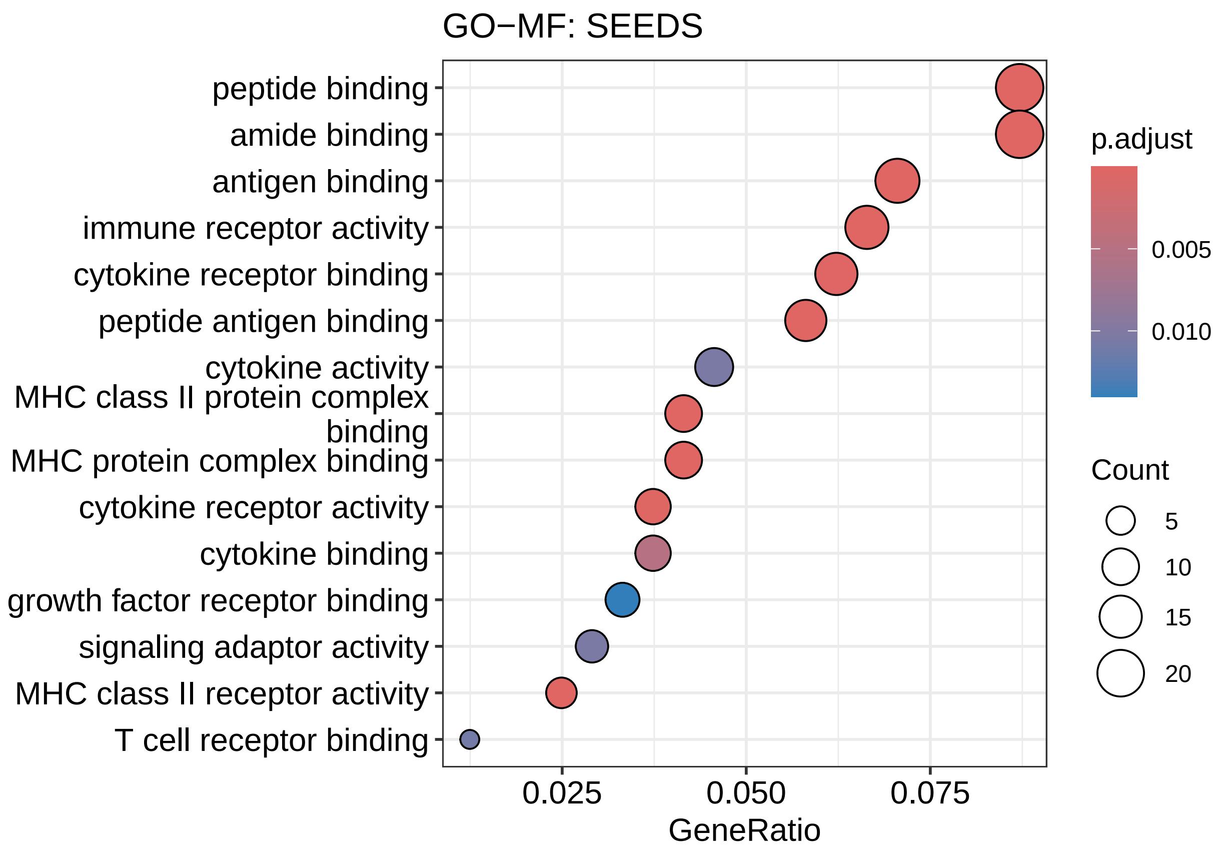

GO−BP: PUTATIVE_TOP100
regulation of T cell activation
leukocyte cell−cell adhesion
leukocyte mediated immunity
regulation of cell−cell adhesion
lymphocyte mediated immunity
regulation of immune effector process
positive regulation of lymphocyte activation
positive regulation of leukocyte activation
regulation of leukocyte cell−cell adhesion
positive regulation of cell activation
positive regulation of cell−cell adhesion
positive regulation of T cell activation
positive regulation of leukocyte cell−cell adhesion
regulation of leukocyte mediated immunity
regulation of lymphocyte mediated immunity
0.300
0.325
0.350
0.375
GeneRatio
p.adjust
4.847272e−37
6.914198e−31
1.382839e−30
2.074259e−30
2.765678e−30
Count
29
31
33
35
37
39

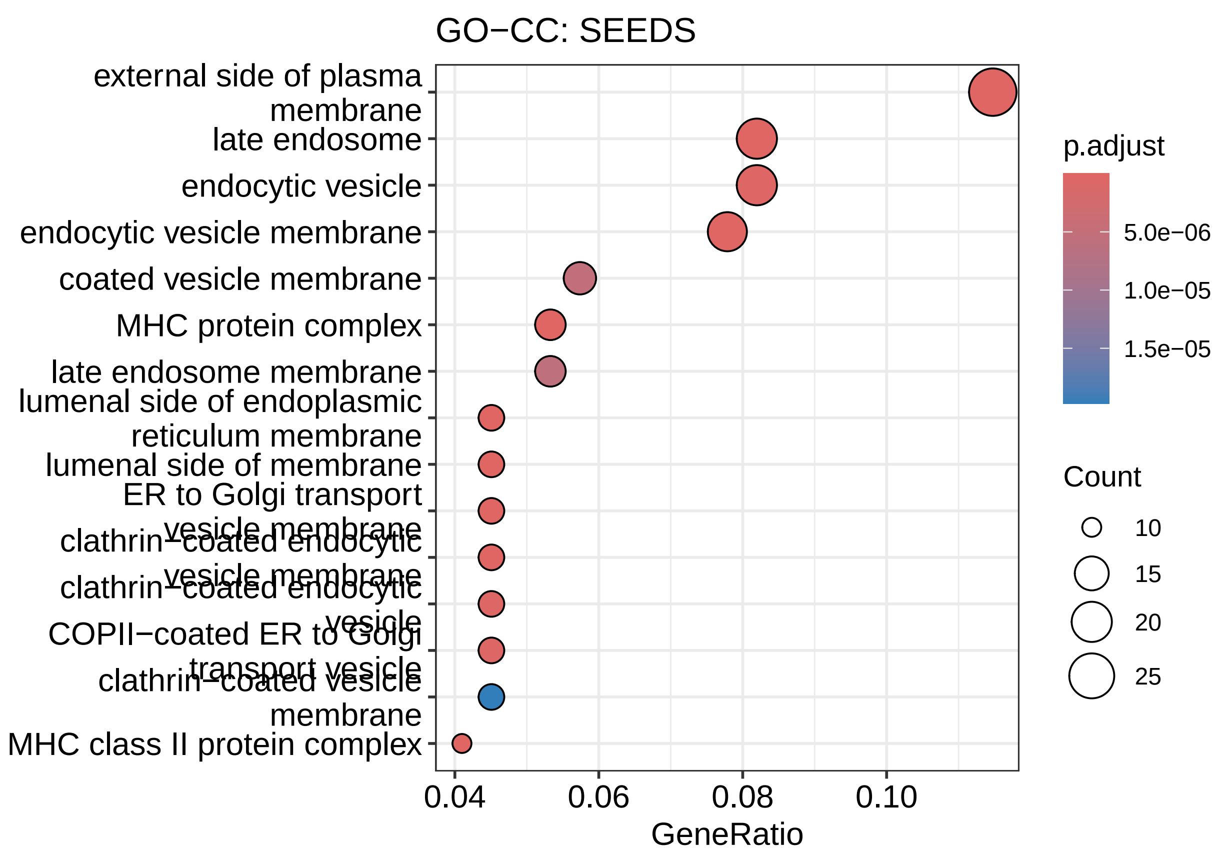

GO−MF: PUTATIVE_TOP100
immune receptor activity
cytokine receptor binding
cytokine receptor activity
cytokine binding
cytokine activity
antigen binding
MHC protein binding
coreceptor activity
C−C chemokine receptor activity
C−C chemokine binding
G protein−coupled chemoattractant receptor activity
chemokine receptor activity
tumor necrosis factor receptor binding
chemokine binding
MHC protein complex binding
0.10
0.15
0.20
0.25
0.30
GeneRatio
p.adjust
5.0e−09
1.0e−08
1.5e−08
2.0e−08
Count
10
15
20
25



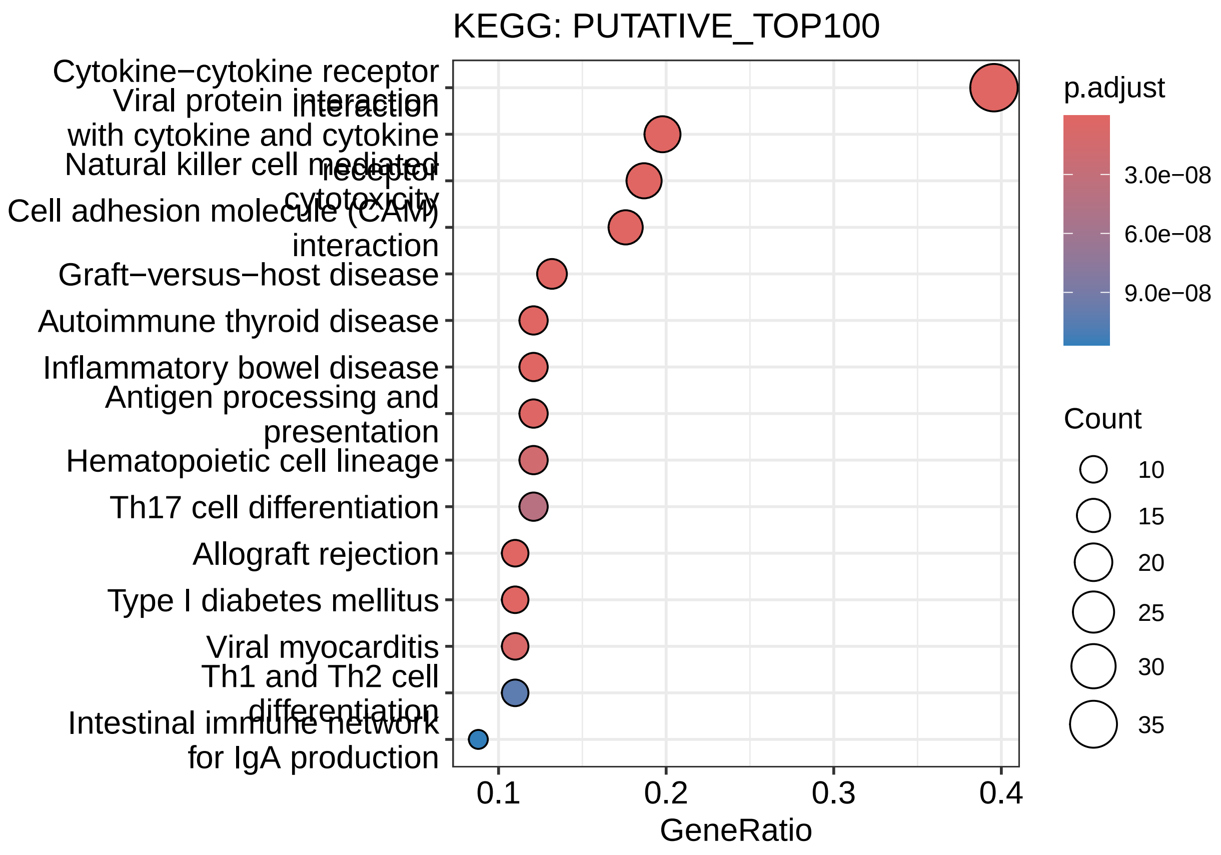
```r
# Seeds GO
run_go_dotplots(entrez_seeds, label = "SEEDS", showCategory = 15, pcut = 0.05)
```


GO−BP: SEEDS
positive regulation of cell activation
positive regulation of leukocyte activation
regulation of cell−cell adhesion
leukocyte cell−cell adhesion
regulation of leukocyte cell−cell adhesion
regulation of T cell activation
positive regulation of lymphocyte activation
positive regulation of cell adhesion
positive regulation of cell−cell adhesion
positive regulation of leukocyte cell−cell adhesion
positive regulation of T cell activation
regulation of immune effector process
leukocyte proliferation
lymphocyte proliferation
mononuclear cell proliferation
0.14
0.16
0.18
GeneRatio
p.adjust
2.500721e−25
3.202494e−16
6.404987e−16
9.607481e−16
1.280997e−15
Count
30
35
40

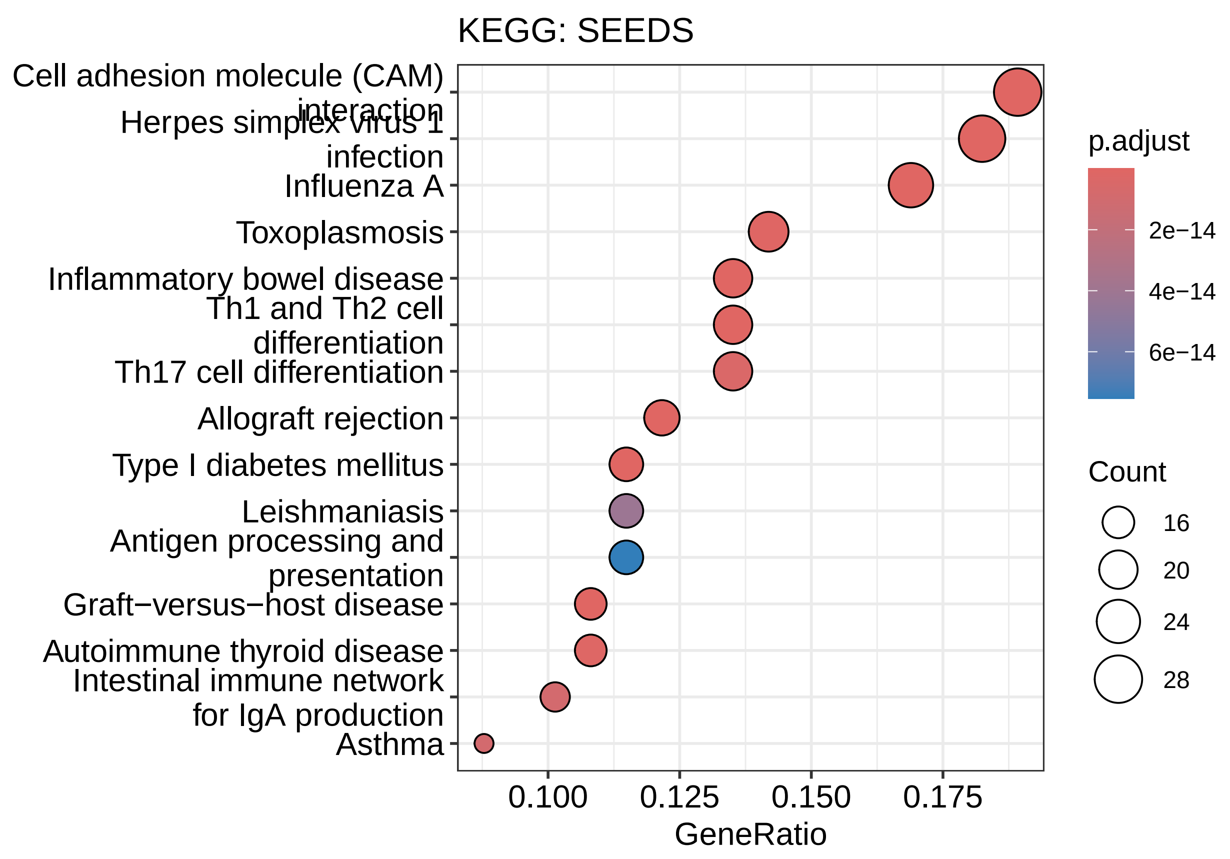

GO−MF: SEEDS
peptide binding
amide binding
antigen binding
immune receptor activity
cytokine receptor binding
peptide antigen binding
cytokine activity
MHC class II protein complex binding
MHC protein complex binding
cytokine receptor activity
cytokine binding
growth factor receptor binding
signaling adaptor activity
MHC class II receptor activity
T cell receptor binding
0.025
0.050
0.075
GeneRatio
p.adjust
0.005
0.010
Count
5
10
15
20



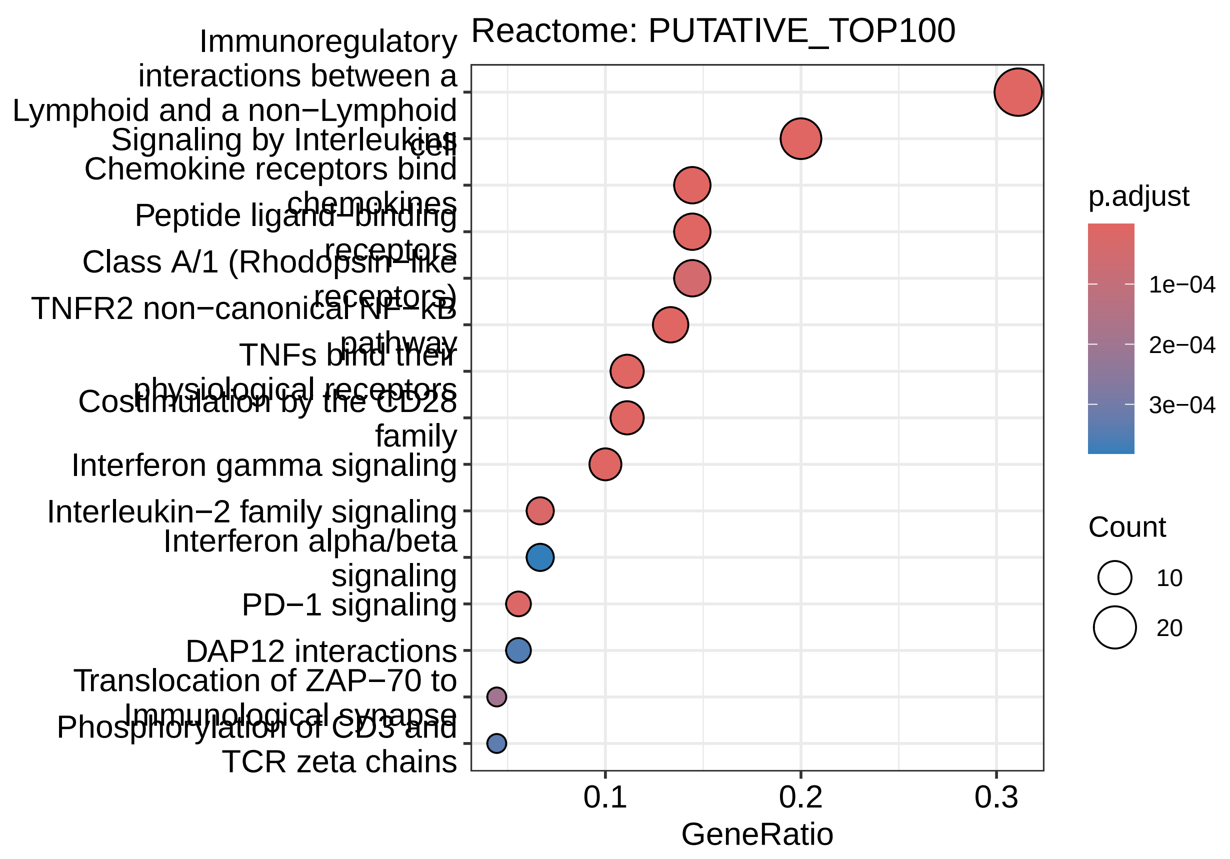
```r
# ---------- KEGG dotplots ----------
run_kegg_dotplot <- function(entrez_vec, label, showCategory = 15, pcut = 0.05) {
  ekegg <- suppressMessages(
    clusterProfiler::enrichKEGG(
      gene         = entrez_vec,
      organism     = "hsa",
      pvalueCutoff = pcut
    )
  )

  message("KEGG terms for ", label, ": ", nrow(as.data.frame(ekegg)))

  p <- enrichplot::dotplot(ekegg, showCategory = showCategory) +
    ggplot2::ggtitle(paste0("KEGG: ", label))

  out_png <- file.path("PART3_outputs/figures", paste0("PART3_dotplot_KEGG_", label, ".png"))
  ggsave(out_png, p, width = 10, height = 6, dpi = 300)
  print(p)
}

run_kegg_dotplot(entrez_putative, "PUTATIVE_TOP100", showCategory = 15, pcut = 0.05)
```



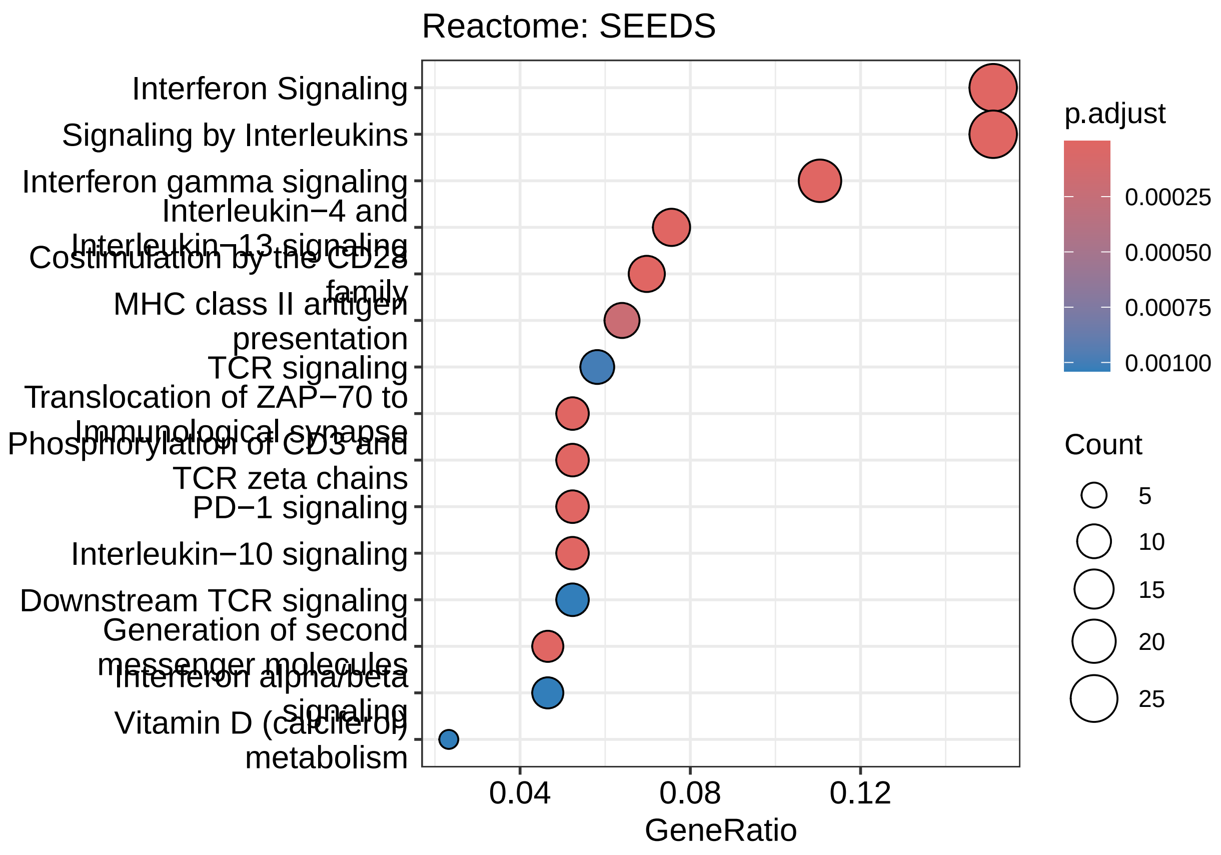
```
run_kegg_dotplot(entrez_seeds,    "SEEDS",          showCategory = 15, pcut = 0.05)
```



```r
# ---------- ReactomePA dotplots ----------
run_reactome_dotplot <- function(entrez_vec, label, showCategory = 15, pcut = 0.05) {
  er <- suppressMessages(
    ReactomePA::enrichPathway(
      gene         = entrez_vec,
      organism     = "human",
      pvalueCutoff = pcut,
      readable     = TRUE
    )
  )

  message("Reactome terms for ", label, ": ", nrow(as.data.frame(er)))

  p <- enrichplot::dotplot(er, showCategory = showCategory) +
    ggplot2::ggtitle(paste0("Reactome: ", label))

  out_png <- file.path("PART3_outputs/figures", paste0("PART3_dotplot_REACTOME_", label, ".png"))
  ggsave(out_png, p, width = 10, height = 6, dpi = 300)
  print(p)
}

run_reactome_dotplot(entrez_putative, "PUTATIVE_TOP100", showCategory = 15, pcut = 0.05)
```



```
run_reactome_dotplot(entrez_seeds,    "SEEDS",          showCategory = 15, pcut = 0.05)
```



Visualization

```
p_overlap <- ggplot(overlap_summary, aes(x = Database, y = Overlap_terms)) +
  geom_col(fill = "steelblue", alpha = 0.8) +
  geom_text(aes(label = Overlap_terms), vjust = -0.5, size = 3.5) +
  theme_minimal(base_size = 12) +
  theme(axis.text.x = element_text(angle = 30, hjust = 1)) +
  labs(
    title = "Overlap of significant EnrichR terms (adjusted p < 0.05)",
    x = "EnrichR library",
    y = "Number of overlapping terms"
  )

ggsave("PART3_outputs/figures/PART3_overlap_counts.png", p_overlap, width = 10, height = 4.5, dpi = 300
print(p_overlap)
```

```
p_jacc <- ggplot(overlap_summary, aes(x = Database, y = Jaccard)) +
  geom_col(fill = "coral", alpha = 0.8) +
  geom_text(aes(label = round(Jaccard, 3)), vjust = -0.5, size = 3.5) +
  theme_minimal(base_size = 12) +
  theme(axis.text.x = element_text(angle = 30, hjust = 1)) +
  labs(
    title = "Jaccard similarity of significant term sets (padj < 0.05)",
    subtitle = "Jaccard = |Overlap| / |Union| after term normalization",
    x = "EnrichR library",
```

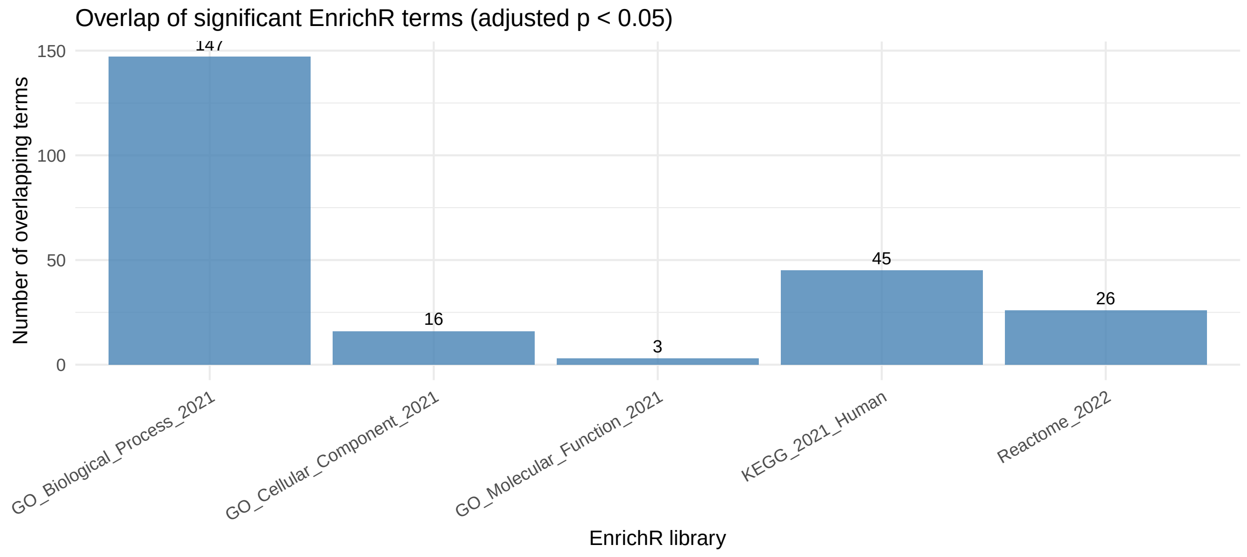


Figure 5: Overlap of significant EnrichR terms between seeds and putative genes

```r
    y = "Jaccard index"
  )

ggsave("PART3_outputs/figures/PART3_overlap_jaccard.png", p_jacc, width = 10, height = 4.5, dpi = 300)
print(p_jacc)
```

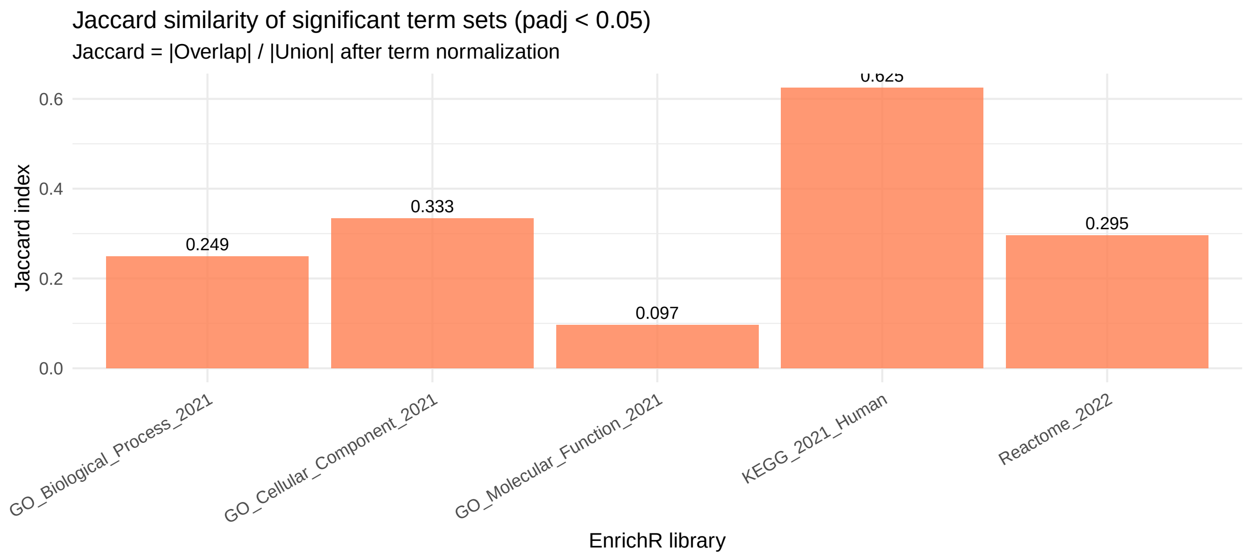


Figure 6: Jaccard similarity of significant term sets

Part 4: Drug Analysis

Setup

```
DISEASE_NAME <- "Multiple Sclerosis"
TOP_GENES_N  <- 20
TOP_DRUGS_N  <- 3

DGIDB_TSV_PATH <- "interactions.tsv"

OUT4 <- "PART4_outputs"
dir.create(OUT4, showWarnings = FALSE, recursive = TRUE)
dir.create(file.path(OUT4, "dgidb"), showWarnings = FALSE, recursive = TRUE)
dir.create(file.path(OUT4, "clinicaltrials"), showWarnings = FALSE, recursive = TRUE)
```

Load Top Genes

```
get_top_putative_genes <- function(n = 20) {
  if (exists("putative_tbl") && is.data.frame(putative_tbl) && all(c("Rank", "Gene") %in% names(putativ
    putative_tbl %>%
      arrange(.data$Rank) %>%
      slice_head(n = n) %>%
      pull(.data$Gene) %>%
      as.character()
  } else if (exists("putative_genes") && is.character(putative_genes)) {
    putative_genes[seq_len(min(n, length(putative_genes)))]
  } else {
    stop("Missing ranked putative genes")
  }
}

top_genes <- unique(str_trim(get_top_putative_genes(TOP_GENES_N)))
top_genes <- top_genes[!is.na(top_genes) & top_genes != ""]

writeLines(top_genes, file.path(OUT4, "top20_putative_genes.txt"))
write_csv(tibble(Rank = seq_along(top_genes), Gene = top_genes),
          file.path(OUT4, "top20_putative_genes.csv"))

message("Top putative genes loaded: n=", length(top_genes))
```

DGIdb Analysis

```
as_logical_robust <- function(x) {
  if (is.logical(x)) return(x)
  x <- as.character(x)
  x <- str_to_lower(str_trim(x))
  x %in% c("true", "t", "1", "yes", "y")
}

load_dgidb_interactions <- function(path) {
  if (!file.exists(path)) stop("DGIdb interactions.tsv not found at: ", path)

  d <- readr::read_tsv(path, show_col_types = FALSE, progress = FALSE)
```

```r
  required <- c("gene_name", "drug_name", "approved")
  missing  <- setdiff(required, names(d))
  if (length(missing) > 0) {
    stop("DGIdb file is missing required columns: ", paste(missing, collapse = ", "))
  }

  d %>%
    mutate(
      gene_name = as.character(.data$gene_name),
      drug_name = as.character(.data$drug_name),
      approved_logical = as_logical_robust(.data$approved)
    ) %>%
    filter(!is.na(.data$gene_name) & .data$gene_name != "",
           !is.na(.data$drug_name) & .data$drug_name != "")
}

dg <- load_dgidb_interactions(DGIDB_TSV_PATH)
dg_approved <- dg %>% filter(.data$approved_logical)
dg_top <- dg_approved %>% filter(.data$gene_name %in% top_genes)

write_csv(dg_top, file.path(OUT4, "dgidb", "dgidb_approved_interactions_top20genes.csv"))

message("DGIdb approved interactions found: n_rows=", nrow(dg_top))
```

Drug Ranking

```r
rank_drugs_by_gene_coverage <- function(dg_top_tbl) {
  if (nrow(dg_top_tbl) == 0) {
    return(tibble(
      drug_name = character(),
      n_genes_top20 = integer(),
      n_interactions = integer(),
      genes_top20 = character()
    ))
  }

  dg_top_tbl %>%
    group_by(.data$drug_name) %>%
    summarise(
      n_genes_top20  = n_distinct(.data$gene_name),
      n_interactions = n(),
      genes_top20    = paste(sort(unique(.data$gene_name)), collapse = ";"),
      .groups = "drop"
    ) %>%
    arrange(desc(.data$n_genes_top20), desc(.data$n_interactions), .data$drug_name)
}

drug_ranking <- rank_drugs_by_gene_coverage(dg_top)

write_csv(drug_ranking, file.path(OUT4, "dgidb", "drug_ranking_by_top20gene_coverage.csv"))

message("Drugs ranked: n=", nrow(drug_ranking))
```

```r
print(drug_ranking)
```

```
## # A tibble: 52 x 4
##    drug_name               n_genes_top20 n_interactions genes_top20
##    <chr>                           <int>          <int> <chr>
##  1 PEGINTERFERON ALFA-2B               3              3 CTLA4;HLA-C;IL21R
##  2 RIBAVIRIN                           3              3 CTLA4;HLA-C;IL21R
##  3 IPILIMUMAB                          1              9 CTLA4
##  4 TREMELIMUMAB                        1              6 CTLA4
##  5 RITUXIMAB                           1              2 FCGR2A
##  6 ABATACEPT                           1              1 CTLA4
##  7 ALDESLEUKIN                         1              1 CD28
##  8 AMOXICILLIN ANHYDROUS               1              1 HLA-C
##  9 ANTI-THYMOCYTE GLOBULIN             1              1 CTLA4
## 10 ATEZOLIZUMAB                        1              1 CTLA4
## # i 42 more rows
```

```r
top_drugs <- drug_ranking %>% slice_head(n = TOP_DRUGS_N) %>% pull(.data$drug_name)
```

Part 5: PROCONSUL Comparison

Setup

```r
OUT5 <- "PART5_outputs"
dir.create(OUT5, showWarnings = FALSE, recursive = TRUE)

stopifnot(file.exists("PROCONSUL.py"))
```

PROCONSUL Functions

```r
run_proconsul <- function(netfile, seeds, m, n_runs, temp, outpath, python_exe, seed = 123) {
  seed_file <- file.path(OUT5, "proconsul_seeds.txt")
  write.table(seeds, seed_file, row.names = FALSE, col.names = FALSE, quote = FALSE)

  cmd <- paste(
    shQuote(python_exe),
    shQuote("PROCONSUL.py"),
    shQuote(netfile),
    shQuote(seed_file),
    "--m", as.integer(m),
    "--n_runs", as.integer(n_runs),
    "--temp", as.numeric(temp),
    "--seed", as.integer(seed),
    "--out", shQuote(outpath)
  )

  system(cmd, intern = FALSE, ignore.stdout = TRUE, ignore.stderr = FALSE)

  if (!file.exists(outpath)) {
    stop("PROCONSUL failed: output not created at ", outpath)
  }
}
```

```r
read_proconsul_rank <- function(path) {
  d <- readr::read_tsv(path, show_col_types = FALSE)
  if (!all(c("rank", "gene") %in% names(d))) {
    stop("Unexpected PROCONSUL output format")
  }
  d %>% arrange(.data$rank) %>% pull(.data$gene) %>% as.character()
}
```

Run PROCONSUL

```r
best_top20 <- get_top_putative_genes(20)
best_top20 <- unique(stringr::str_trim(best_top20))
best_top20 <- best_top20[!is.na(best_top20) & best_top20 != ""]

writeLines(best_top20, file.path(OUT5, "bestperformer_top20.txt"))
```

```r
# NOTA: Questo chunk è impostato con eval=FALSE perché PROCONSUL richiede molto tempo.
# I risultati sono già salvati in PART5_outputs/proconsul_*_temp1.tsv
netfile_best <- paste0(best_network, "_network.txt")

M_PRO <- 20
N_RUNS_PRO <- 50
TEMP_PRO <- 1.0

pro_out <- file.path(OUT5, paste0("proconsul_", best_network, "_temp1.tsv"))

run_proconsul(
  netfile = netfile_best,
  seeds = final_seeds,
  m = M_PRO,
  n_runs = N_RUNS_PRO,
  temp = TEMP_PRO,
  outpath = pro_out,
  python_exe = python_exe,
  seed = 123
)
```

```r
# Carica i risultati già calcolati
pro_out <- file.path(OUT5, paste0("proconsul_", best_network, "_temp1.tsv"))

if (!file.exists(pro_out)) {
  # Cerca file PROCONSUL esistenti
  pro_files <- list.files(OUT5, pattern = "^proconsul_.*_temp1\\.tsv$", full.names = TRUE)
  if (length(pro_files) > 0) {
    pro_out <- pro_files[1]
    message("Using existing PROCONSUL output: ", pro_out)
  } else {
    stop("PROCONSUL output not found. Run the algorithm first or set eval=TRUE in the previous chunk.")
  }
}

pro_rank <- read_proconsul_rank(pro_out)
pro_top20 <- pro_rank[seq_len(min(20, length(pro_rank)))]
```

```r
pro_top20 <- unique(stringr::str_trim(pro_top20))
pro_top20 <- pro_top20[!is.na(pro_top20) & pro_top20 != ""]

writeLines(pro_top20, file.path(OUT5, "proconsul_temp1_top20.txt"))
```

Compare Results

```r
overlap <- intersect(best_top20, pro_top20)
jaccard <- if (length(union(best_top20, pro_top20)) == 0) NA_real_ else
  length(overlap) / length(union(best_top20, pro_top20))

writeLines(overlap, file.path(OUT5, "overlap_genes_top20.txt"))

cmp_tbl <- tibble::tibble(
  Method_A = paste0("BestPerformer_", best_algo, "_", best_network),
  Method_B = paste0("PROCONSUL_temp1_", best_network),
  TopK = 20,
  BestTop20_n = length(best_top20),
  ProconsulTop20_n = length(pro_top20),
  Overlap_n = length(overlap),
  Jaccard = jaccard,
  Overlap_genes = paste(overlap, collapse = ";")
)

readr::write_csv(cmp_tbl, file.path(OUT5, "TOP20_overlap_best_vs_proconsul_temp1.csv"))

message("PROCONSUL done. Outputs written to: ", OUT5)

print(cmp_tbl)
```

```
## # A tibble: 1 x 8
##   Method_A         Method_B  TopK BestTop20_n ProconsulTop20_n Overlap_n Jaccard
##   <chr>            <chr>    <dbl>       <int>            <int>     <int>   <dbl>
## 1 BestPerformer_D~ PROCONS~    20          20               20        19   0.905
## # i 1 more variable: Overlap_genes <chr>
```

Summary

This R Markdown document contains the complete BNM project analysis for Multiple Sclerosis, including:

- Part 1: Interactome construction and disease gene mapping
- Part 2: Cross-validation of gene prioritization methods
- Part 3: Functional enrichment analysis of putative genes
- Part 4: Drug-gene interaction and clinical trial analysis
- Part 5: Comparison with PROCONSUL algorithm

All outputs are saved in their respective directories for further analysis