

R codes of analysis gene

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1 File: analysis__data.1.R

```
## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl")
## -----
## -----
## -----
## ===== Run block =====
gse <- meta$Accession[1]
set.sig.wd(gse)
meta.df <- decomp_tar2txt()
## -----
meta.df <- dplyr::mutate(
  meta.df,
  treatment = stringr::str_extract(
    file, "(?<=D7-).*(?=_RNA-seq)"
  ),
  group = treatment,
  sample = stringr::str_extract(
    file, "(?<=_).*(?=_RNA-seq)"
  )
)
## -----
gene.anno.tmp <- data.table::fread(meta.df$file[1]) %>%
  dplyr::select(grep("Symbol|Ensembl|reference sequence", colnames(.))) %>%
```

```

dplyr::mutate(ref = `Locus on reference sequence`,
              chr = stringr::str_extract(ref, "^chr[0-9]{1,}(?:=)"),
              seq.st = stringr::str_extract(ref, "(?<=)[0-9]{1,}(?=-)"),
              seq.st = as.integer(seq.st),
              seq.end = stringr::str_extract(ref, "(?<=)[0-9]{1,}$"),
              seq.end = as.integer(seq.end),
              eff.len = abs(seq.st - seq.end)) %>%
dplyr::relocate(`Ensembl Gene ID`, `eff.len`) %>%
dplyr::as_tibble()
## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(12, 2))
## add group
dge.list <- re.sample.group(dge.list, meta.df)
## add annotation
dge.list <- anno.into.list(dge.list, gene.anno.tmp, "Ensembl Gene ID")
## -----
## fpkm to tpm
dge.list <- fpkm_log2tpm(dge.list)
## -----
group. <- dge.list$samples$group
## design
design <- model.matrix(~ 0 + group.)
## contrast
contr.matrix <- limma::makeContrasts(
  ## treat with CH223191
  treat_ch.vs.contr = group.CH - group.C,
  ## treat with StemRegenin1
  treat_sr.vs.contr = group.SR1 - group.C,
  levels = design
)
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix,
                      min.count = 0.0001, voom = F)
## -----
## save
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

2 File: analysis_data.10.R

```

## R
## meta

```

```

## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = c("ensembl_exon_id", "go_id"))
## -----

gse <- meta$Accession[10]
set.sig.wd(gse)
## -----

info <- GEOquery::getGEO(gse)
## -----

meta.df.raw <- Biobase::phenoData(info[[1]]) %>%
  Biobase::pData() %>%
  dplyr::as_tibble()
## -----

meta.df <- dplyr::select(meta.df.raw, title) %>%
  dplyr::mutate(sample = title,
                group = gsub("_[0-9]{1,}$", "", sample),
                group = gsub("U87_shC_", "", group))
## -----

group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## contrast
contr.matrix <- limma::makeContrasts(
  treat_ficz.vs.contr = group.FICZ100nM - group.DMSO,
  treat_kyna.vs.contr = group.KynA50uM - group.DMSO,
  levels = design
)
## -----

## show expression dataset
# exprs <- Biobase::assayData(info[[1]])$exprs
# print(head(exprs))
## -----

# genes <- fit$genes %>%
#   dplyr::as_tibble()
## -----

res <- limma_downstream.eset(info[[1]], design, contr.matrix)
## -----

## ===== Run block =====
res <- lapply(res, dplyr::mutate,
              ensembl = stringr::str_extract(gene_assignment, "ENS[A-Z][0-9]*"),
              symbol = stringr::str_extract(gene_assignment,

```

```

      "(?<= |^)[A-Z]{1,}[0-9]{0,2}[A-Z]{1,}[0-9]{0,2}[A-Z]{0,}[0-9]{0,3}(?= |$)") %>%
  lapply(dplyr::relocate, ensembl, symbol)
## -----
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

3 File: analysis_data.11.R

```

## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = c("ensembl_exon_id", "go_id"))
## -----
## -----
gse <- meta$Accession[11]
set.sig.wd(gse)
## -----
info <- GEOquery::getGEO(gse)
## -----
meta.df.raw <- Biobase::phenoData(info[[1]]) %>%
  Biobase::pData() %>%
  dplyr::as_tibble()
## -----
meta.df <- dplyr::select(meta.df.raw, title) %>%
  dplyr::mutate(sample = title,
               group = gsub("_[0-9]{1,}$", "", sample),
               group = gsub("U87_", "", group))
## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## contrast
contr.matrix <- limma::makeContrasts(
  treat_i3ca.vs.contr = group.I3CA50uM - group.DMSO,
  levels = design
)
## -----
## show expression dataset
exprs <- Biobase::assayData(info[[1]])$exprs
## -----
res <- limma_downstream.eset(info[[1]], design, contr.matrix)
## -----

```

```

res <- lapply(res, dplyr::mutate,
              ensembl = stringr::str_extract(gene_assignment, "ENS[A-Z][0-9]*"),
              symbol = stringr::str_extract(gene_assignment,
              "(?<= |^)[A-Z]{1,}[0-9]{0,2}[A-Z]{1,}[0-9]{0,2}[A-Z]{0,}[0-9]{0,3}(?= |$)")) %>%
  lapply(dplyr::relocate, ensembl, symbol)
## -----
## ===== Run block =====
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

4 File: analysis_data.12.R

```

## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = c("ensembl_exon_id", "go_id"))
## -----
## -----
gse <- meta$Accession[12]
set.sig.wd(gse)
## -----
info <- GEOquery::getGEO(gse)
## -----
meta.df.raw <- Biobase::phenoData(info[[1]]) %>%
  Biobase::pData() %>%
  dplyr::as_tibble()
## -----
meta.df <- dplyr::select(meta.df.raw, title) %>%
  dplyr::mutate(sample = title,
               group = gsub("_[0-9]{1,}$", "", sample),
               group = gsub("^.*U87_", "", group))
## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## -----
## contrast
contr.matrix <- limma::makeContrasts(
  treat_hpp.vs.contr = group.HPP40uM - group.DMSO,
  treat_i3p.vs.contr = group.I3P40uM - group.DMSO,
  treat_pp.vs.contr = group.PP40uM - group.DMSO,
  levels = design

```

```

)
## -----
## show expression dataset
# exprs <- Biobase::assayData(info[[1]])$exprs
## -----
res <- limma_downstream.eset(info[[1]], design, contr.matrix)
## -----
res <- lapply(res, dplyr::mutate,
              ensembl = stringr::str_extract(gene_assignment, "ENS[A-Z][0-9]*"),
              symbol = stringr::str_extract(gene_assignment,
              "(?<= |^)[A-Z]{1,}[0-9]{0,2}[A-Z]{1,}[0-9]{0,2}[A-Z]{0,}[0-9]{0,3}(?= |$)")) %>%
  lapply(dplyr::relocate, ensembl, symbol)
## -----
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

5 File: analysis_data.13.R

```

## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = c("ensembl_exon_id", "go_id"))
## -----
## -----
gse <- meta$Accession[13]
set.sig.wd(gse)
## -----
info <- GEOquery::getGEO(gse)
## -----
meta.df.raw <- Biobase::phenoData(info[[1]]) %>%
  Biobase::pData() %>%
  dplyr::as_tibble()
## -----
meta.df <- dplyr::select(meta.df.raw, title) %>%
  dplyr::mutate(sample = title,
               group = gsub("_[0-9]{1,}$", "", sample),
               group = gsub("^.*U87_", "", group))
## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## -----

```

```
## contrast
contr.matrix <- limma::makeContrasts(
  ## treat with L-trp
#   il4i1_ahrKO.vs.ahrKO_1 = group.IL4I1_shAHR1 - group.C_shAHR1,
#   il4i1_ahrKO.vs.ahrKO_2 = group.IL4I1_shAHR2 - group.C_shAHR2,
  ahrKO_1.vs.control = group.C_shAHR1 - group.C_shC,
  ahrKO_2.vs.control = group.C_shAHR2 - group.C_shC,
  levels = design
)
## -----
## show expression dataset
# exprs <- Biobase::assayData(info[[1]])$exprs
## -----
res <- limma_downstream.eset(info[[1]], design, contr.matrix)
## -----
res <- lapply(res, dplyr::mutate,
  ensembl = stringr::str_extract(gene_assignment, "ENS[A-Z][0-9]*"),
  symbol = stringr::str_extract(gene_assignment,
    "(?<=|^)[A-Z]{1,}[0-9]{0,2}[A-Z]{1,}[0-9]{0,2}[A-Z]{0,}[0-9]{0,3}(?<=|$)") %>%
    lapply(dplyr::relocate, ensembl, symbol)
## -----
## ===== Run block =====
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)
```

6 File: analysis_data.16.R

```
## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = c("ensembl_exon_id", "go_id"))
## -----
## -----
gse <- meta$Accession[16]
set.sig.wd(gse)
## -----
info <- GEOquery::getGEO(gse)
## -----
meta.df.raw <- Biobase::phenoData(info[[1]]) %>%
  Biobase::pData() %>%
  dplyr::as_tibble()
```



```

## -----
meta.df <- dplyr::select(meta.df.raw, title) %>%
  dplyr::mutate(sample = title,
               group = gsub(" [0-9]{1,}$", "", sample),
               group = gsub("-", "_", group))

## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)

## -----
## contrast
contr.matrix <- limma::makeContrasts(
  treat.vs.control = group.Co_culture - group.Single_culture,
  levels = design
)

## -----
## show expression dataset
# exprs <- Biobase::assayData(info[[1]])$exprs
## -----
res <- limma_downstream.eset(info[[1]], design, contr.matrix)
## -----
res <- lapply(res, dplyr::mutate,
             ensembl = stringr::str_extract(SPOT_ID.1, "ENS[A-Z][0-9]*"),
             symbol = stringr::str_extract(SPOT_ID.1,
             "(?<=\\() [A-Z]{1,}[0-9]{0,2}[A-Z]{1,}[0-9]{0,2}[A-Z]{0,}[0-9]{0,3}(?=\\))")) %>%
  lapply(dplyr::relocate, ensembl, symbol)
## -----
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

7 File: analysis_data.17.R

```

## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = c("ensembl_exon_id", "go_id"))
## -----
check <- 0
n <- 0
while(check == 0){
  n <- n + 1
  check <- try(gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl",

```

```

ex.attr = c("go_id", "refseq_mrna")),

      silent = T)
if(class(check)[1] == "try-error"){
  print(check)
  check <- 0
}else{
  check <- 1
}
cat("##", "Try...", n, "\n")
}

## -----
gse <- meta$Accession[17]
set.sig.wd(gse)
## -----
meta.df <- decomp_tar2txt()
## -----
meta.df <- dplyr::mutate(meta.df, sample = gsub("^GSM[^_]*_", "", file),
                        sample = gsub("\\.txt$", "", sample),
                        group = gsub("_60_S.*_pool.*$", "", sample))
## -----
## format
raw <- lapply(meta.df$file, data.table::fread) %>%
  lapply(dplyr::distinct, tracking_id, .keep_all = T) %>%
  lapply(dplyr::rename, fpkm = 6) %>%
  lapply(dplyr::mutate, fpkm = as.numeric(fpkm))
## filter NA
nas <- lapply(raw, dplyr::filter, is.na(fpkm)) %>%
  lapply(`[[`, "tracking_id") %>%
  unlist(use.names = F) %>%
  unique()
raw <- lapply(raw, dplyr::filter, !tracking_id %in% all_of(nas))
## -----
gene.anno.tmp <- dplyr::select(raw[[1]], 1:2)
## write into disk
mapply(write_tsv, raw, meta.df$file)
## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(1, 6))
## add group
dge.list <- re.sample.group(dge.list, meta.df)
## -----
## add annotation

```

```

dge.list <- anno.into.list(dge.list, gene.anno.tmp, "tracking_id")
## -----
dge.log2tpm(dge.list)
## -----
group. <- dge.list$samples$group
## design
design <- model.matrix(~ 0 + group.)
## contrast
## -----
contr.matrix <- limma::makeContrasts(
  treat_bap.vs.contr = group.MCF10AT1_BA_P - group.MCF10AT1_NT,
  treat_bpa.vs.contr = group.MCF10AT1_BPA - group.MCF10AT1_NT,
  treat_overlay.vs.contr = group.MCF10AT1_BPAlus_Ba_P - group.MCF10AT1_NT,
  levels = design
)
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix,
  min.count = 0.0001, voom = F)
## -----
## ===== Run block =====
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

8 File: analysis_data.18.R

```

## R
## meta
## -----
gse <- meta$Accession[18]
set.sig.wd(gse)
## -----
list.files(pattern = "\\..gz$") %>%
  R.utils::gunzip()
## -----
## ===== Run block =====
raw <- data.table::fread("GSE130234_processed_data.txt") %>%
  dplyr::as_tibble()
## -----
mapply(2:ncol(raw), colnames(raw)[2:ncol(raw)],
  FUN = function(col, name){
    df <- raw[, c(1, col)]
    write_tsv(df, paste0(name, "_counts.tsv"))
  }
)

```

```

    })

## -----
meta.df <- data.table::data.table(
  file = list.files(pattern = "_counts.tsv$")
) %>%
  dplyr::mutate(sample = gsub("_tagcount_counts.tsv", "", file),
               sample = gsub("-", "_", sample),
               group = gsub("_rep.", "", sample))

## -----
## annotation
gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl",
                              attr = c("ensembl_gene_id", "hgnc_symbol", "refseq_mrna"))

## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(1, 2))
## add group
dge.list <- re.sample.group(dge.list, meta.df)
## add annotation
dge.list <- anno.into.list(dge.list, gene.anno, "refseq_mrna")
## -----
# keeps <- !duplicated(dge.list$genes$ensembl_gene_id) | grepl("^NM_", dge.list$genes$refseq_mrna)
# ## filter...
# dge.list <- edgeR::`[.DGEList`(dge.list, keeps, , keep.lib.sizes = F)
## -----
group. <- dge.list$samples$group
## design
design <- model.matrix(~ 0 + group.)
## contrast
contr.matrix <- limma::makeContrasts(
  treat_sga315.vs.contr = group.LOV_SGA315 - group.LOV,
  treat_sga360.vs.contr = group.LOV_SGA360 - group.LOV,
  levels = design
)
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix)
## -----
## remove mRNA sequences of non-coding proteins
res <- lapply(res, dplyr::filter, !grepl("^NR_", refseq_mrna)) %>%
  lapply(dplyr::relocate, ensembl_gene_id, hgnc_symbol) %>%
  ## remove duplicated genes
  lapply(dplyr::distinct, ensembl_gene_id, .keep_all = T)
## -----

```

```
## distribution
# exprs <- limma_downstream(dge.list, group., design, contr.matrix, get_normed.exprs = T)
## -----
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)
```

9 File: analysis_data.19.R

```
## R
## meta
## -----
gse <- meta$Accession[19]
set.sig.wd(gse)
## -----
info <- GEOquery::getGEO(gse)
## -----
meta.df.raw <- Biobase::phenoData(info[[1]]) %>%
  Biobase::pData() %>%
  dplyr::as_tibble()
## -----
meta.df <- dplyr::select(meta.df.raw, title) %>%
  dplyr::mutate(sample = title,
               group = gsub("^HepG2 ", "", sample),
               group = gsub(" ", "_", group))
## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## -----
## contrast
contr.matrix <- limma::makeContrasts(
  treat_mc3_1h.vs.control = group.MC3_1_h - group.NT,
  treat_mc3_24h.vs.control = group.MC3_24_h - group.NT,
  levels = design
)
## -----
## show expression dataset
# exprs <- Biobase::assayData(info[[1]])$exprs
## -----
# res <- limma_downstream.eset(info[[1]], design, contr.matrix)
```

10 File: analysis_data.2.R

```
## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl")
## -----
## -----
## -----
gse <- meta$Accession[2]
set.sig.wd(gse)
meta.df <- decomp_tar2txt()
## -----
meta.df <- dplyr::filter(meta.df, !grepl("peaks.txt", file)) %>%
  dplyr::mutate(
    treatment = stringr::str_extract(
      file, "(?<=D7-).*(?=_RNA-seq)"
    ),
    group = treatment,
    sample = stringr::str_extract(
      file, "(?<=_).*(?=_RNA-seq)"
    )
  )
## -----
gene.anno.tmp <- data.table::fread(meta.df$file[1]) %>%
  dplyr::select(grep("Symbol|Ensembl|reference sequence", colnames(.))) %>%
  dplyr::mutate(ref = `Locus on reference sequence`,
    chr = stringr::str_extract(ref, "^chr[0-9]{1,}(?=:)"),
    seq.st = stringr::str_extract(ref, "(?<=:)[0-9]{1,}(?=-)"),
    seq.st = as.integer(seq.st),
    seq.end = stringr::str_extract(ref, "(?<=-)[0-9]{1,}$"),
    seq.end = as.integer(seq.end),
    eff.len = abs(seq.st - seq.end)) %>%
  dplyr::relocate(`Ensembl Gene ID`, `eff.len`) %>%
  dplyr::as_tibble()
## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(12, 2))
## add group
dge.list <- re.sample.group(dge.list, meta.df)
## add annotation
dge.list <- anno.into.list(dge.list, gene.anno.tmp, "Ensembl Gene ID")
```

```

## fpkm to tpm
dge.list <- fpkm_log2tpm(dge.list)
## -----
## group
group. <- dge.list$samples$group
## design
design <- model.matrix(~ 0 + group.)
## contrast
contr.matrix <- limma::makeContrasts(
  ## treat with CH223191
  treat_ch.vs.contr = group.CH - group.C,
  ## treat with StemRegenin1
  treat_sr.vs.contr = group.SR1 - group.C,
  levels = design
)
## -----
## ===== Run block =====
res <- limma_downstream(dge.list, group., design, contr.matrix,
  min.count = 0.0001, voom = F)
## -----
## save
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

11 File: analysis_data.20.R

```

## R
## meta
## -----
gse <- meta$Accession[20]
set.sig.wd(gse)
## -----
list.files(pattern = "\\\\.gz$") %>%
  R.utils::gunzip()
## -----
raw <- data.table::fread("GSE104869_tablecounts.txt") %>%
  dplyr::as_tibble()
## -----
## remove the duplicated geneid
raw <- dplyr::mutate(raw, Geneid = gsub("\\.[0-9]$", "", Geneid)) %>%
  dplyr::distinct(Geneid, .keep_all = T)
## -----

```

```

mapply(2:ncol(raw), colnames(raw)[2:ncol(raw)],
       FUN = function(col, name){
         df <- raw[, c(1, col)]
         write_tsv(df, paste0(name, "_counts.tsv"))
       })

## -----
meta.df <- data.table::data.table(
  file = list.files(pattern = "_counts.tsv$")
) %>%
  dplyr::mutate(sample = gsub("_counts.tsv", "", file),
               sample = gsub(" ", "_", sample),
               sample = gsub("\\\\+", "_plus_", sample),
               group = gsub("[0-9]$", "", sample))

## -----
gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl",
                              attr = c("ensembl_gene_id", "hgnc_symbol", "refseq_mrna"))

## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(1, 2))

## add group
dge.list <- re.sample.group(dge.list, meta.df)

## add annotation
dge.list <- anno.into.list(dge.list, gene.anno, "hgnc_symbol")

## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)

## -----
## contrast
contr.matrix <- limma::makeContrasts(
  treat_R.vs.control = group.501Mel_R - group.501Mel_Ctrl,
  treat_T.vs.control = group.501Mel_T - group.501Mel_Ctrl,
  levels = design
)

## -----
res <- limma_downstream(dge.list, group., design, contr.matrix)

## -----
res <- lapply(res, dplyr::relocate, ensembl_gene_id, hgnc_symbol) %>%
  lapply(dplyr::filter, !is.na(ensembl_gene_id))

## -----
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```


12 File: analysis_data.21.R

```
## R
## meta
## -----
gse <- meta$Accession[21]
set.sig.wd(gse)
## -----
meta.df <- decomp_tar2txt()
## -----
meta.df <- dplyr::mutate(
  meta.df,
  sample = stringr::str_extract(file, "(?<=_).*(?=\\.count)"),
  sample = gsub("-", "_", sample),
  group = gsub("_[0-9]$", "", sample)
)
## -----
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl",
#                                attr = c("ensembl_gene_id", "hgnc_symbol", "refseq_mrna"))
## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(1, 2))
## add group
dge.list <- re.sample.group(dge.list, meta.df)
## add annotation
dge.list <- anno.into.list(dge.list, gene.anno, "hgnc_symbol")
## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## -----
## contrast
contr.matrix <- limma::makeContrasts(
  treat_d1.vs.control = group.TCDD_d1 - group.DMSO_d1,
  treat_d2.vs.control = group.TCDD_d2 - group.DMSO_d2,
  levels = design
)
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix)
## -----
res <- lapply(res, dplyr::relocate, ensembl_gene_id, hgnc_symbol) %>%
  lapply(dplyr::filter, !is.na(ensembl_gene_id))
## -----
## ===== Run block =====
```

```
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)
```

13 File: analysis_data.22.R

```
## R
## meta
## -----
## ===== Run block =====
gse <- meta$Accession[22]
set.sig.wd(gse)
## -----
```

14 File: analysis_data.23.R

```
## R
## meta
## -----
gse <- meta$Accession[23]
set.sig.wd(gse)
## -----
list.files(pattern = "\\\\.gz") %>%
  lapply(R.utils::gunzip)
## -----
raw <- data.table::fread("GSE116637_counts.txt") %>%
  dplyr::mutate(Geneid = gsub("-[0-9]{1,}$", "", Geneid)) %>%
  dplyr::distinct(Geneid, .keep_all = T) %>%
  dplyr::as_tibble()
## -----
lapply(7:ncol(raw), function(col){
  df <- raw[, c(1:6, col)]
  file <- colnames(raw)[col]
  write_tsv(df, paste0(file, "_counts.tsv"))
})
## -----
meta.df <- data.table::data.table(
  file = list.files(pattern = "_counts.tsv$")
) %>%
  dplyr::mutate(sample = gsub("_counts.tsv", "", file),
               group = gsub("_[0-9]$", "", sample),
               cell = stringr::str_extract(group, "^[^_]{1,}"),
```

```

        agonist = stringr::str_extract(group, "[^_]{1,}$"))
cell.type <- meta.df$cell %>%
  unique()
agonist.type <- meta.df$agonist %>%
  unique()
## -----
gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl",
                              attr = c("ensembl_gene_id", "hgnc_symbol", "refseq_mrna"))
## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(1, 7))
## add group
dge.list <- re.sample.group(dge.list, meta.df)
## add annotation
dge.list <- anno.into.list(dge.list, gene.anno, "hgnc_symbol")
## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## -----
contr <- data.table::data.table(
  treat = c("3MC", "GNF"),
  control = "DMSO"
)
contr <- lapply(cell.type, function(cell){
  dplyr::mutate(contr,
    .treat = treat,
    treat = paste0("group.", cell, "_", treat),
    control = paste0("group.", cell, "_", control),
    contr = paste0(treat, " - ", control),
    name = paste0("treat_", cell, "_", .treat, ".vs.control"))
})
contr <- data.table::rbindlist(contr)
args <- lapply(contr$contr, function(text){
  parse(text = text)
})
names(args) <- contr$name
args$levels <- design
## contrast matrix
contr.matrix <- do.call(limma::makeContrasts, args)
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix)
## -----

```

```
## ===== Run block =====
res <- lapply(res, dplyr::relocate, ensembl_gene_id, hgnc_symbol) %>%
  lapply(dplyr::filter, !is.na(ensembl_gene_id))
## save
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)
```

15 File: analysis_data.24.R

```
## R
## meta
## -----
gse <- meta$Accession[24]
set.sig.wd(gse)
## -----
```

16 File: analysis_data.25.R

```
## R
## meta
## -----
## ===== Run block =====
gse <- meta$Accession[25]
set.sig.wd(gse)
## -----
```

17 File: analysis_data.26.R

```
## R
## meta
## -----
gse <- meta$Accession[26]
set.sig.wd(gse)
## -----
meta.df <- decomp_tar2txt()
## -----
raw <- lapply(meta.df$file, data.table::fread)
## -----
names(raw) <- meta.df$file
raw <- lapply(raw, dplyr::rename, symbol = 4, counts = 6) %>%
  lapply(dplyr::relocate, symbol, counts) %>%
```

```

lapply(dplyr::distinct, symbol, .keep_all = T)
## save as tibble
mapply(raw, names(raw), FUN = function(df, file){
  write_tsv(df, file)
})
## -----
## ===== Run block =====
meta.df <- dplyr::mutate(meta.df,
  sample = gsub("^GSM[0-9]{1,}_|_RPKM.txt", "", file),
  group = gsub("[0-9]{1,}-", "", sample),
  group = gsub("-", "_", group),
  .group = group,
  block = stringr::str_extract(.group, "[^_]{1,}"),
  group = gsub("[^_]{1,}", "AML", .group),
  cell = stringr::str_extract(group, "[^_]*(?=_)"),
  agonist = stringr::str_extract(group, "(?<=_)\\.*$"))
cell.type <- meta.df$cell %>%
  unique()
agonist.type <- meta.df$agonist %>%
  unique()
## -----
gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl",
  attr = c("ensembl_gene_id", "hgnc_symbol", "refseq_mrna"))
## -----
dge.list <- edgeR::readDGE(meta.df$file, columns = c(1, 2))
## add group
dge.list <- re.sample.group(dge.list, meta.df)
## add annotation
dge.list <- anno.into.list(dge.list, gene.anno, "hgnc_symbol")
## -----
group. <- meta.df$group
design <- model.matrix(~ 0 + group.)
## -----
contr <- data.table::data.table(
  treat = agonist.type[3:length(agonist.type)],
  control = "DMSO"
)
contr <- lapply(cell.type, function(cell){
  dplyr::mutate(contr,
    .treat = treat,
    treat = paste0("group.", cell, "_", treat),

```

```

        control = paste0("group.", cell, "_", control),
        contr = paste0(treat, " - ", control),
        name = paste0("treat_", cell, "_", .treat, ".vs.control"))
})
contr <- data.table::rbindlist(contr) %>%
  dplyr::filter(treat %in% colnames(design))
args <- lapply(contr$contr, function(text){
  parse(text = text)
})
names(args) <- contr$name
args$levels <- design
## -----
## contrast matrix
contr.matrix <- do.call(limma::makeContrasts, args)
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix, block = meta.df$block)
## -----
res <- lapply(res, dplyr::relocate, ensembl_gene_id, hgnc_symbol) %>%
  lapply(dplyr::filter, !is.na(ensembl_gene_id))
## save
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

18 File: analysis__data.27.R

```

## R
## meta
## -----
## ===== Run block =====
gse <- meta$Accession[27]
set.sig.wd(gse)
## -----

```

19 File: analysis__data.28.R

```

## R
## meta
## -----
## ===== Run block =====
gse <- meta$Accession[28]
set.sig.wd(gse)

```

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

20 File: analysis_data.3.R

```
## R
## meta
## -----
## annotation
gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl")
## -----
## -----
## -----

gse <- meta$Accession[3]
set.sig.wd(gse)
# meta.df <- decomp_tar2txt()
## get infoma...
info <- GEOquery::getGEO(gse)
## -----
## download data respectively
get_gsm.data(info)
## -----
# control (scramble) siRNA + DMSO (GSM5579231, GSM5579232) vs
# control (scramble) siRNA + TCDD (GSM5579233)
meta.df <- data.table::data.table(
  file.xlsx = list.files(pattern = "^GSM557923[1-3]{1}_.*\\.xlsx")
) %>%
  dplyr::mutate(group = rep(c("control", "treatment"), c(2, 1)),
               file = gsub("\\.xlsx$", ".txt", file.xlsx),
               sample = c("control_1", "control_2", "treat"))
## -----
## format data
mapply(meta.df$file.xlsx, meta.df$file,
       FUN = function(xlsx, txt){
         df <- readxl::read_xlsx(xlsx) %>%
           dplyr::relocate(ENSEMBL, `Total exon reads`)
         write_tsv(df, txt)
       })
## -----
gene.anno.tmp <- data.table::fread(meta.df$file[1]) %>%
  dplyr::select(ENSEMBL, Name, Chromosome, `Exon length`)
## -----
```

```

dge.list <- edgeR::readDGE(meta.df$file)
## group
dge.list <- re.sample.group(dge.list, meta.df)
## annotation
dge.list <- anno.into.list(dge.list, gene.anno.tmp, "ENSEMBL")
## -----
group. <- dge.list$samples$group
## design
design <- model.matrix(~ 0 + group.)
## contrast
contr.matrix <- limma::makeContrasts(
  treat.vs.contr = group.treatment - group.control,
  levels = design
)
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix)
## save
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

21 File: analysis_data.4.R

```

## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl")
## -----
## -----
## -----
gse <- meta$Accession[4]
set.sig.wd(gse)
## unzip
list.files(pattern = "\\..gz") %>%
  lapply(R.utils::gunzip)
## -----
## ===== Run block =====
df <- readxl::read_excel("GSE183606_differentially_expressed_genes.xlsx") %>%
  ## rutaecarpin vs contral
  dplyr::filter(abs(`Log2FC(Rutaecar/Control)`) > 0.3, Padjust < 0.05)
write_tsv(df, "treat.vs.contr_results.tsv")

```


22 File: analysis_data.5.R

```
## R
## meta
## -----
## annotation
# attr <- list.attr.biomart()
# check <- 0
# n <- 0
# while(check == 0){
#   n <- n + 1
#   check <- try(gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = "go_id"),
#               silent = T)
#   if(class(check)[1] == "try-error"){
#     print(check)
#     check <- 0
#   }else{
#     check <- 1
#   }
#   cat("##", "Try...", n, "\n")
# }
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = "go_id")
## -----
## -----
## -----
gse <- meta$Accession[5]
set.sig.wd(gse)
## unzip
list.files(pattern = "\\\\.gz") %>%
  lapply(R.utils::gunzip)
## -----
## ===== Run block =====
res <- data.table::fread("GSE188657_processed_data_files.txt") %>%
  dplyr::filter(abs(log2FoldChange) > 0.3, qValue < 0.05) %>%
  dplyr::as_tibble()
# treat.vs.contr:: AhR antagonist (StemRegenin 1) vs DMSO
```

23 File: analysis_data.6.R

```
## R
## meta
```

```

## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = "go_id")
## -----
## -----
gse <- meta$Accession[6]
set.sig.wd(gse)
## -----
info <- GEOquery::getGEO(gse)
## -----
info <- info[1]
get_gsm.data(info)
## -----
list.files(pattern = ".tsv.gz$", recursive = T, full.names = T) %>%
  sapply(function(path){
    system(paste("mv", path, "-t ."))
  })
## -----
list.files(pattern = ".tsv.gz$", recursive = T, full.names = T) %>%
  sapply(R.utils::gunzip)
## -----
## metadata
meta.df <- data.table::fread("metadata.csv", header = F) %>%
  dplyr::rename(sample = 1, anno = 2) %>%
  dplyr::mutate(group = ifelse(grepl("Untreated", anno), "control", "treatment"),
    time = stringr::str_extract(anno, "(?<=_)[0-9]{1,}(?=<hr_)" ),
    group = paste0(group, "_", time),
    file = paste0(sample, ".tsv"))
## -----
## separate annotation
gsm.file <- list.files(pattern = "abundance.tsv$") %>%
  sapply(function(file){
    df <- data.table::fread(file) %>%
      dplyr::mutate(ensembl.v = stringr::str_extract(target_id,
        "(?<=\\|)ENSG[0-9]{1,}[^\\|]{1,}(?=\\|)",
        ensembl = stringr::str_extract(ensembl.v, "^ENSG[0-9]{1,}") ) %>%
      dplyr::relocate(ensembl, tpm) %>%
      dplyr::distinct(ensembl, .keep_all = T)
    file <- stringr::str_extract(file, "^GSM[0-9]{1,}(?=_)")
    write_tsv(df, paste0(file, ".tsv"))
  })

```

```

        return(file)
    })
    ## -----
    gene.anno.tmp <- data.table::fread(meta.df$file[1]) %>%
      dplyr::select(ensembl, ensembl.v, eff_length)
    ## -----
    dge.list <- edgeR::readDGE(meta.df$file)
    ## add group
    dge.list <- re.sample.group(dge.list, meta.df)
    ## add annotation
    dge.list <- anno.into.list(dge.list, gene.anno, "ensembl_gene_id")
    ## log2 tpm
    dge.list$counts <- apply(dge.list$counts, 2,
                           function(vec){
                             log2(vec + 1)
                           })
    ## -----
    group. <- dge.list$samples$group
    ## design
    design <- model.matrix(~ 0 + group.)
    ## -----
    ## contrast
    contr.matrix <- limma::makeContrasts(
      treat.vs.contr_6 = group.treatment_6 - group.control_6,
      treat.vs.contr_18 = group.treatment_18 - group.control_18,
      treat.vs.contr_72 = group.treatment_72 - group.control_72,
      levels = design
    )
    ## -----
    res <- limma_downstream(dge.list, group., design, contr.matrix,
                          min.count = 0.0001, voom = F)
    ## -----
    ## ===== Run block =====
    mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)

```

24 File: analysis_data.7.R

```

## R
## meta
## -----
## annotation

```

```

# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = "go_id")
## -----
## -----
## -----

gse <- meta$Accession[7]
set.sig.wd(gse)
## -----

list.files(pattern = "txt\\.gz$", recursive = T, full.names = T) %>%
  sapply(R.utils::gunzip)
## -----

raw.res <- list.files(pattern = "processed") %>%
  lapply(data.table::fread) %>%
  lapply(function(df){
    df[1:2, ]
  })
## -----

raw.res <- lapply(raw.res, function(df){
  mutate.df <- data.table::data.table(
    ncol = 1:ncol(df),
    contrast = unlist(df[1, ], use.names = F),
    type = unlist(df[2, ], use.names = F)
  )
  return(mutate.df)
})
## -----

form.res <- lapply(raw.res, function(df){
  dplyr::filter(df, contrast == "" | grepl(" vs ", contrast))
}) %>%
  lapply(by_group_as_list, colnames = "contrast")
## the annotation col
ex.anno.cal <- form.res[[1]][[1]]
## contrast col
form.res <- lapply(form.res, function(lst){
  lst[[1]] <- NULL
  lst
})
## -----

raw.res <- list.files(pattern = "processed") %>%
  lapply(data.table::fread, skip = 2, header = F)
## -----

res <- mapply(form.res, raw.res,

```

```

SIMPLIFY = F,
FUN = function(form, raw){
  ## convert data.table to tibble
  raw <- dplyr::as_tibble(raw)
  lst <- lapply(form, raw = raw,
    FUN = function(entry, raw){
      col <- c(1:4, entry$ncol)
      ## colnames of data.frame
      col.name <- c(ex.anno.cal$type, "log2FC", "p-value", "q-value")
      ## extract column
      df <- raw[, col]
      colnames(df) <- col.name
      ## filter data
      df <- dplyr::filter(df, abs(log2FC) > 0.3, `q-value` < 0.05)
      return(df)
    })
  })

## -----
res <- unlist(res, recursive = F)
## -----
contrast.entry <- names(res)
contrast <- contrast.entry[c(3, 8, 9, 10)]
res <- res[names(res) %in% contrast]
## -----
## ===== Run block =====
mapply(res, names(res),
  FUN = function(df, names){
    write_tsv(df, paste0(names, "_results.tsv"))
  })

```

25 File: analysis_data.8.R

```

## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = "go_id")
## -----
## -----
## -----
gse <- meta$Accession[8]

```

```

set.sig.wd(gse)
## -----
res.raw <- list.files(pattern = "Results.xlsx") %>%
  readxl::read_xlsx()
## -----
res.raw <- dplyr::select(res.raw, 1:4, contains(c("Log2FC", "pAdj")))
## -----
res <- reshape2::melt(res.raw, id.vars = colnames(res.raw)[1:4],
  variable.name = "type",
  value.name = "value") %>%
  dplyr::as_tibble() %>%
  dplyr::mutate(contrast = gsub("_Log2FC|_pAdj", "", type),
    stat = stringr::str_extract(type, "(?<=_)[^_]{1,}$")) %>%
  dplyr::select(-type) %>%
  by_group_as_list("contrast")
## -----
res <- lapply(res, tidyr::spread, key = stat, value = value)
## -----
res <- lapply(res, dplyr::filter, abs(Log2FC) > 0.3, pAdj < 0.05)
## -----
res <- lapply(res, dplyr::mutate, Ensembl = gsub("\\.[0-9]{1,}$", "", GeneID)) %>%
  lapply(dplyr::relocate, Ensembl, ID)
## -----
contrast <- names(res)[c(1, 2, 3, 4)]
res <- res[names(res) %in% contrast]
## -----
## ===== Run block =====
mapply(res, names(res),
  FUN = function(df, names){
    write_tsv(df, paste0(names, "_results.tsv"))
  })

```

26 File: analysis__data.9.R

```

## R
## meta
## -----
## annotation
# gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl", ex.attr = "go_id")
## -----
## -----

```

```

## -----
gse <- meta$Accession[9]
set.sig.wd(gse)
## -----
list.files(pattern = "\\..gz$") %>%
  R.utils::gunzip()
## -----
raw <- list.files(pattern = "\\..tab$") %>%
  data.table::fread() %>%
  dplyr::as_tibble()
## -----
lapply(2:ncol(raw), function(ncol){
  file <- paste0(colnames(raw)[ncol], ".tsv")
  write_tsv(raw[, c(1, ncol)], file)
})
## -----
meta.df <- data.table::data.table(
  file = list.files(pattern = "[0-9]\\..tsv$")
) %>%
dplyr::mutate(
  sample = gsub("\\..tsv$", "", file),
  group = gsub("_[0-9]$", "", sample),
  group = gsub("-", "_", group)
)
## -----
dge.list <- edgeR::readDGE(meta.df$file)
## group
dge.list <- re.sample.group(dge.list, meta.df)
## annotation
dge.list <- anno.into.list(dge.list, gene.anno, "ensembl_gene_id")
## -----
group. <- dge.list$samples$group
## design
design <- model.matrix(~ 0 + group.)
## -----
## contrast
contr.matrix <- limma::makeContrasts(
  treat_4.vs.contr = group.ahr_lna_4_6 - group.nc_lna_6,
  treat_7.vs.contr = group.ahr_lna_7_6 - group.nc_lna_6,
  levels = design
)

```

```
## -----
res <- limma_downstream(dge.list, group., design, contr.matrix)
## -----
## ===== Run block =====
mapply(res, paste0(names(res), "_results.tsv"), FUN = write_tsv)
```

27 File: from_dataset.R

```
## R
## metadata
## path <- "~/operation/geo_db/ahr_sig"
## -----
## ===== Run block =====
meta <- data.table::fread("series.csv") %>%
  dplyr::filter(grepl("Expression", `Series Type`),
               grepl("Homo sapiens", Taxonomy)) %>%
  dplyr::as_tibble()
## -----
## Use wget to download data
apply(dplyr::mutate(meta, seq = 1:nrow(meta)), 1,
      function(vec){
        cat("[Info] Downloading seq of", vec[["seq"]], "\n")
        gse <- vec[["Accession"]]
        gse.dir <- gsub("[0-9]{3}$", "nnn", gse)
        ftp <- paste0("ftp://ftp.ncbi.nlm.nih.gov/geo/series/", gse.dir, "/", gse, "/suppl/")
        system(paste("wget -np -m", ftp))
      })
## -----
## test for download data
## -----
# file <- list.files(pattern = test)
# info.t <- GEOquery::getGEO(filename = file)
# ## -----
# df <- Biobase::phenoData(info.t)
# name.sample <- Biobase::sampleNames(df)
# ## -----
# sample <- GEOquery::getGEO(name.sample[1])
# sample.df <- GEOquery::Table(sample)
# ## -----
```


28 File: gather_results.R

```
## R
## gather analysis results
setwd("~/operation/geo_db/ahr_sig/")
## -----
all_results <- list.files(pattern = "_results.tsv",
                          recursive = T,
                          full.names = T) %>%

  data.frame() %>%
  dplyr::rename(file = 1) %>%
  dplyr::mutate(filename = stringr::str_extract(file, "[^/]*$"),
                contrast = stringr::str_extract(filename, "^.*(?= _results)"),
                series = stringr::str_extract(file, "(?<=/)GSE[0-9]{1,}(?=/)"))
## -----
## cell, treat.left, treat.right
all_series <- all_results$series %>%
  unique()
## -----
lst.sum <- lapply(all_series, function(gse){
  info <- try_do("GEOquery::getGEO(gse)", envir = environment())
  info <- lapply(info,
                function(obj){
                  obj <- Biobase::experimentData(obj)
                  obj@other$summary
                })
})
names(lst.sum) <- all_series
## -----
lst.sum <- lapply(lst.sum, `[`, 1) %>%
  data.table::data.table() %>%
  dplyr::rename(summary = 1) %>%
  dplyr::mutate(Accession = all_series, summary = unlist(summary)) %>%
  dplyr::as_tibble()
## -----
meta.summary <- merge(meta, lst.sum, by = "Accession", all.y = T) %>%
  dplyr::as_tibble()
write_tsv(meta.summary, "meta.summary.tsv")
## -----
gene.anno <- anno.gene.biomart("hsapiens_gene_ensembl",
                              attr = c("ensembl_gene_id", "hgnc_symbol"))
## -----
```

```

screened.genes <- lapply(all_results$file, data.table::fread) %>%
  lapply(function(df){
    df <- df %>%
      dplyr::rename(ensembl = 1, symbol = 2)
    ## if number > n, filter out the rest
    n <- 1000
    if(nrow(df) > n){
      ## col of adjust p.value
      adj.p <- colnames(df) %>%
        .[grepl("adj|q-value", ., ignore.case = T)]
      df <- dplyr::rename(df, adj.p = paste0(adj.p)) %>%
        dplyr::arrange(adj.p) %>%
        ## get top n
        head(n = n) %>%
        dplyr::relocate(ensembl, symbol)
    }
    df <- dplyr::select(df, 1:2)
    return(df)
  }) %>%
  data.table::rbindlist() %>%
  dplyr::filter(!is.na(ensembl) & !is.na(symbol) & symbol != "") %>%
  dplyr::filter(ensembl %in% all_of(gene.anno$ensembl_gene_id)) %>%
  # dplyr::mutate(symbol = gsub("\\.[0-9]$", "", symbol)) %>%
  dplyr::distinct() %>%
  dplyr::as_tibble()

## -----
## AHR targets were retrieved from the Transcription Factor Target Gene Database
## <http://tfbsdb.systemsbio.net/>
tf.db <- data.table::fread("TFTGD_ahr_targes.tsv") %>%
  dplyr::mutate(symbol = ifelse(grepl("^V_", Motif),
                                stringr::str_extract(Motif, "(?<=^V_)[^_]{1,}"),
                                stringr::str_extract(Motif, "^[^_]{1,}"))) %>%
  dplyr::distinct(symbol)

## -----
tf.db <- dplyr::filter(gene.anno, hgnc_symbol %in% all_of(tf.db$symbol)) %>%
  dplyr::select(ensembl_gene_id, hgnc_symbol) %>%
  distinct() %>%
  dplyr::rename(ensembl = 1, symbol = 2)

## -----
## ===== Run block =====
## merge genes from 'screened.genes' and 'tf.db'

```

```
merge.scre_tf <- dplyr::bind_rows(screened.genes, tf.db) %>%  
  dplyr::distinct()  
write_tsv(merge.scre_tf, "merge.scre_tf.tsv")  
## -----
```