

expression_heatmaps

Rtpw

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Goal: make heatmaps that will be used to visualize intestine gene expression or ELT-2 transcriptional dependence for each stage to compliment deepTools plots generated by David.

The heatmaps will be broken out into the following categories:

- ELT-2 bound, intestine enriched
- ELT-2 bound, intestine non-enriched
- ELT-2 not bound, intestine enriched
- ELT-2 not bound, intestine non-enriched

the rows within each category will be arranged from high to low for elt-2 (-) log2FoldChange.

Load libraries

```
library(tidyverse)

## -- Attaching packages ----- tidyverse 1.3.1 --
## v ggplot2 3.3.5      v purrr   0.3.4
## v tibble  3.1.6      v dplyr   1.0.8
## v tidyr   1.2.0      v stringr 1.4.0
## v readr   2.1.2      v forcats 0.5.1

## Warning: package 'tidyr' was built under R version 4.1.2
## Warning: package 'readr' was built under R version 4.1.2
## Warning: package 'dplyr' was built under R version 4.1.2

## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()     masks stats::lag()

library(ComplexHeatmap)

## Loading required package: grid

## =====
## ComplexHeatmap version 2.8.0
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
## Github page: https://github.com/jokergoo/ComplexHeatmap
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
##
## If you use it in published research, please cite:
```

```
## Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
## genomic data. Bioinformatics 2016.
##
## The new InteractiveComplexHeatmap package can directly export static
## complex heatmaps into an interactive Shiny app with zero effort. Have a try!
##
## This message can be suppressed by:
## suppressPackageStartupMessages(library(ComplexHeatmap))
## =====
library(circlize)

## Warning: package 'circlize' was built under R version 4.1.2
## =====
## circlize version 0.4.14
## CRAN page: https://cran.r-project.org/package=circlize
## Github page: https://github.com/jokergoo/circlize
## Documentation: https://jokergoo.github.io/circlize\_book/book/
##
## If you use it in published research, please cite:
## Gu, Z. circlize implements and enhances circular visualization
## in R. Bioinformatics 2014.
##
## This message can be suppressed by:
## suppressPackageStartupMessages(library(circlize))
## =====
source("../elt2_paper_functions.R")
```

Analysis and plotting function

```
expression_heatmaps <- function(stage, chip.df, gut_categories, gut_res, elt2_res){
heatmap_df <- chip.df %>%
  mutate(binding_status = case_when(is.na(IDR_mean) ~ "not_bound", !is.na(IDR_mean) ~ "bound")) %>%
  select(WBGeneID, gene_name, binding_status) %>%
  left_join(gut_categories %>% select(WBGeneID, intestine_expression), by = "WBGeneID") %>%
  mutate(intestine_expression = case_when(intestine_expression == "enriched" ~ "enriched",
                                          intestine_expression != "enriched" ~ "not_enriched",
                                          is.na(intestine_expression) ~ "not_enriched")) %>%
  left_join(res_embryoGFPplus_vs_embryoGFPminus_ashr_shrunk %>%
    select(WBGeneID, gutFC = log2FoldChange, gutPadj = padj), by = "WBGeneID"
  ) %>%
  left_join(res_elt2D_v_wt_ashr_shrunk %>% select(WBGeneID, elt2FC = log2FoldChange),
    by = "WBGeneID"
  ) %>%
  replace_na(list(gutFC = 0, elt2FC = 0)) %>%
  mutate(row_split = case_when(
    binding_status == "bound" & intestine_expression == "enriched" ~ "classA",
    binding_status == "bound" & intestine_expression == "not_enriched" ~ "classB",
    binding_status == "not_bound" & intestine_expression == "enriched" ~ "classC",
    binding_status == "not_bound" & intestine_expression == "not_enriched" ~ "classD"
  )) %>%
  arrange(desc(elt2FC))
```

```

heatmap_mat <- heatmap_df %>%
  select(WBGeneID, gutFC, elt2FC) %>%
  column_to_rownames(var = "WBGeneID") %>%
  as.matrix()

gut_FC <- Heatmap(name = paste(stage, "_gut_FC", sep = ""),
  heatmap_mat[, "gutFC"],
  show_row_names = FALSE,
  cluster_rows = FALSE,
  cluster_columns = FALSE,
  row_title_rot = 0,
  col = colorRamp2(c(min(heatmap_df$gutFC), 0, max(heatmap_df$gutFC)), c("blue", "white", "darkgold"),
  row_split = heatmap_df$row_split,
  width = unit(0.5, "in"),
  border_gp = gpar(col = "black", lty = 1))

elt2_FC <- Heatmap(name = "elt2_FC",
  heatmap_mat[, "elt2FC"],
  show_row_names = FALSE,
  cluster_rows = FALSE,
  cluster_columns = FALSE,
  row_title_rot = 0,
  col = colorRamp2(c(min(heatmap_df$elt2FC), 0, max(heatmap_df$elt2FC)), c("darkgreen", "white", "deeppink"),
  row_split = heatmap_df$row_split,
  width = unit(0.5, "in"),
  border_gp = gpar(col = "black", lty = 1))
#darkorchid4
#deeppink
gut_FC + elt2_FC
}

```

Embryo Stage

Load data

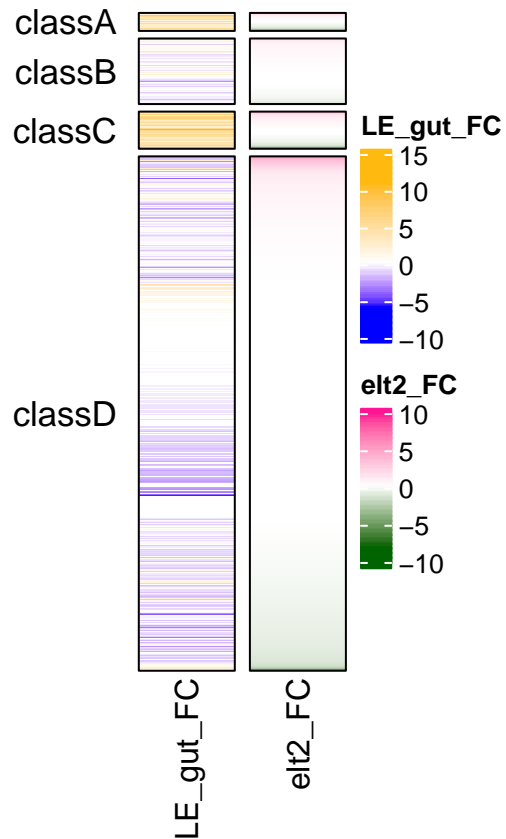
```

embryo_intestine_gene_categories <- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/intestine_gene_categories.csv")
res_embryoGFPplus_vs_embryoGFPminus_ashr_shrunk <- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/res_embryoGFPplus_vs_embryoGFPminus_ashr_shrunk.csv")
elt2_regulated_gene_sets <- read_csv("../03_elt2_RNAseq/03_output/elt2_regulated_gene_sets.csv")
res_elt2D_v_wt_ashr_shrunk <- read_csv("../03_elt2_RNAseq/03_output/res_elt2D_v_wt_ashr_shrunk.csv")
LE.promoters.hilo <- read.table("../04_promoters/03_output/LE.promoters.hilo.tsv") %>% rownames_to_column()

res_embryoGFP_alHyp_greater <- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/res_embryoGFP_alHyp_greater.csv")

embryo_expression_heatmaps <- expression_heatmaps("LE", LE.promoters.hilo, embryo_intestine_gene_categories)
embryo_expression_heatmaps

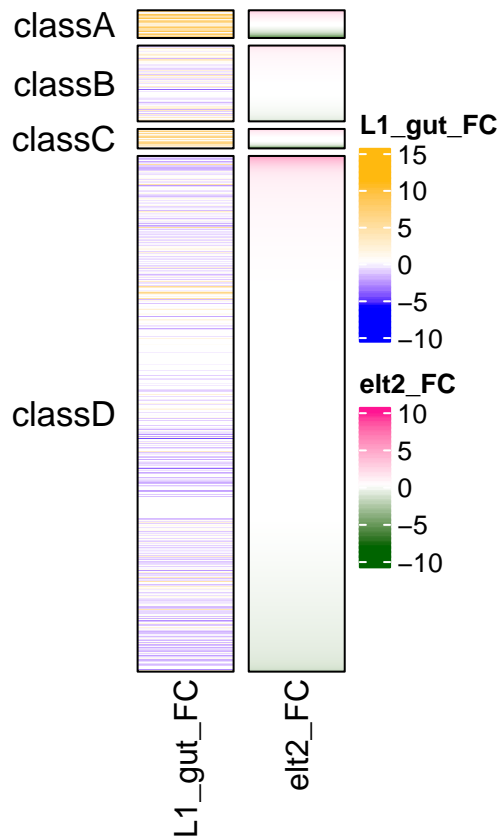
```



```
myPDFplot(embryo_expression_heatmaps, name = "embryo_expression_heatmaps", height = 4, width = 3, plotd
## pdf
## 2
```

L1 stage

```
L1_intestine_gene_categories<- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/intestine_gene_c
res_L1GFPplus_vs_L1GFPminus_ashr_shrunk <- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/pair
L1.promoters.hilo <- read.table("../04_promoters/03_output/L1.promoters.hilo.tsv") %>% rownames_to_c
res_L1GFP_alHyp_greater <- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/res_L1GFP_alHyp_grea
L1_expression_heatmaps <- expression_heatmaps("L1",L1.promoters.hilo, L1_intestine_gene_categories, res
L1_expression_heatmaps
```



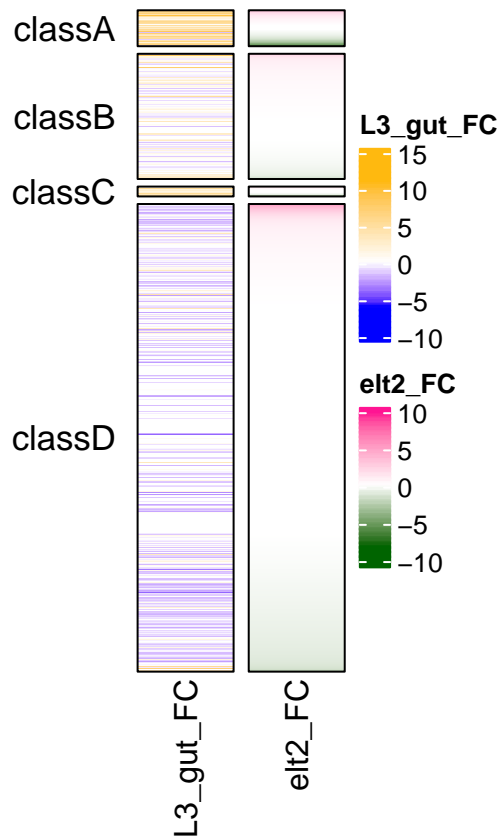
```
myPDFplot(L1_expression_heatmaps, name = "L1_expression_heatmaps", height = 4, width = 3, plotdir = "..")
```

```
## pdf
## 2
```

L3 stage

```
L3_intestine_gene_categories<- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/intestine_gene_categories.csv")
res_L3GFPplus_vs_L3GFPminus_ashr_shrunk <- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/pairwise_differences/res_L3GFPplus_vs_L3GFPminus_ashr_shrunk.csv")
L3.promoters.hilo <- read.table("../04_promoters/03_output/L3.promoters.hilo.tsv") %>% rownames_to_column()
res_L3GFP_alHyp_greater <- read_csv("../02_emb_L1_L3_intestine_RNAseq/03_output/res_L3GFP_alHyp_greater.csv")

L3_expression_heatmaps <- expression_heatmaps("L3",L3.promoters.hilo, L3_intestine_gene_categories, res_L3GFPplus_vs_L3GFPminus_ashr_shrunk, res_L3GFP_alHyp_greater)
L3_expression_heatmaps
```



```
myPDFplot(L3_expression_heatmaps, name = "L3_expression_heatmaps", height = 4, width = 3, plotdir = "..")
```

```
## pdf
## 2
```

Session info

```
sessionInfo()
```

```
## R version 4.1.0 (2021-05-18)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Catalina 10.15.7
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] grid      stats      graphics  grDevices  utils      datasets  methods
## [8] base
##
## other attached packages:
## [1] circlize_0.4.14      ComplexHeatmap_2.8.0 forcats_0.5.1
## [4] stringr_1.4.0        dplyr_1.0.8          purrr_0.3.4
```

```

## [7] readr_2.1.2          tidyr_1.2.0          tibble_3.1.6
## [10] ggplot2_3.3.5         tidyverse_1.3.1
##
## loaded via a namespace (and not attached):
## [1] httr_1.4.2            bit64_4.0.5          vroom_1.5.7
## [4] jsonlite_1.8.0        foreach_1.5.2        modelr_0.1.8
## [7] assertthat_0.2.1      highr_0.9            stats4_4.1.0
## [10] cellranger_1.1.0      yaml_2.3.5           pillar_1.7.0
## [13] backports_1.4.1       glue_1.6.2           digest_0.6.29
## [16] RColorBrewer_1.1-3    rvest_1.0.2          colorspace_2.0-3
## [19] htmltools_0.5.2       pkgconfig_2.0.3      GetoptLong_1.0.5
## [22] broom_0.8.0           haven_2.4.3          scales_1.2.0
## [25] tzdb_0.3.0            generics_0.1.2       IRanges_2.26.0
## [28] ellipsis_0.3.2        withr_2.5.0          BiocGenerics_0.38.0
## [31] cli_3.2.0             magrittr_2.0.3       crayon_1.5.1
## [34] readxl_1.4.0          evaluate_0.15        fs_1.5.2
## [37] fansi_1.0.3           doParallel_1.0.17    xml2_1.3.3
## [40] Cairo_1.5-15          tools_4.1.0          hms_1.1.1
## [43] GlobalOptions_0.1.2   lifecycle_1.0.1      matrixStats_0.61.0
## [46] S4Vectors_0.30.2     munsell_0.5.0        reprex_2.0.1
## [49] cluster_2.1.3         compiler_4.1.0       rlang_1.0.2
## [52] iterators_1.0.14      rstudioapi_0.13      rjson_0.2.21
## [55] rmarkdown_2.13        gtable_0.3.0         codetools_0.2-18
## [58] DBI_1.1.2            R6_2.5.1             lubridate_1.8.0
## [61] knitr_1.38           bit_4.0.4            fastmap_1.1.0
## [64] utf8_1.2.2           clue_0.3-60          shape_1.4.6
## [67] stringi_1.7.6         parallel_4.1.0       vctrs_0.4.0
## [70] png_0.1-7            dbplyr_2.1.1         tidyselect_1.1.2
## [73] xfun_0.30

```