RWC23_ELT2_Regulated_Genes

RTPW

4/13/2020

Install Packages

```
# if (!requireNamespace("BiocManager", quietly = TRUE))
#     install.packages("BiocManager")
# BiocManager::install()
# BiocManager::install("biomaRt")
# install.packages("tidyverse")
# install.packages("readxl")
# BiocManager::install("ComplexHeatmap")
# install.packages("matrixStats")
# install.packages("pheatmap")
# install.packages("RVAideMemoire")
# install.packages("dendextend")
# install.packages("binom")
```

Load Package Libraries

```
library(biomaRt)
library(tidyverse)
library(readxl)
library(ComplexHeatmap)
library(matrixStats)
library(pheatmap)
library(RVAideMemoire)
library(dendextend)
library(binom)
library(circlize)
```

To do

- cluster set assignments derived from heatmap do not match when plotted back to the heatmap

Background and Rationale

ELT-2 is the C. elegans intestine master regulator. Deletion of ELT-2 leads to a larval lethal phenotype, and expression of ELT-2 in non-intestine tissue induces an intestine fate.

This documet will generate plots to address the questions outlined below.

For genes differentially expressed during elt-2 (-) and/or elt-7(-):

1) which expression pattern clusters associate with ELT-2 binding?
2) which expression pattern clusters associate with ELT-2 binding categories?

- For all genes
- For only genes bound by ELT-2

3) Which expression pattern clusters associate with intestine expression? (MA plot for each expression set)

- For all genes
- For genes only bound by ELT-2

For clusters of transcription factors (TFs) differentially expressed during elt-2 (-) and/or elt-7(-):

1) which transcription factor clusters associate with ELT-2 binding?
2) which transcription factor clusters associate with ELT-2 binding categories

- for all TFs
- For only TFs bound by ELT-2

3) which transcription factor clusters associate with intestine expression?

- for all
- for only ELT-2 bound

Data

I will integrate two RNA-seq experiments and a ChIP-seq experiments.

The first is a set of RNA-seq experiments in L1 stage worms (Dineen and Nishimura, 2018). They were collected from the following genotypes, all in the L1 stage:

- wildtype (wt)
- elt-7 deleted (elt7D)
- elt-2 deleted (elt2D)
- combination fo elt-7 and elt-2 deleted (elt2Delt7D)

The purpose of including elt-7 and elt-2/elt-7 double deletion is because these two transcription factors have overlapping functionality. Deletion of elt-7 alone does not have a phenotype, but deletion of elt-7 in combination with elt-2 has an enhanced lethal phenotype of just elt-2 alone.

The second RNA-seq experiment is from FACS sorted L1 stage intestine cells. This data is unpublished.

The ChIP-seq experiments are performed against ELT-2 and are from the following developmental stages:

- late embryo (LE)
- L1
- L3

They were collected as part of the modENCODE consortium and were processed by David King. He has provided gene mapping of ELT-2 targets and categories of ELT-2 binding. The ELT-2 binding categories are as follows:

- Not changing
- Larval
- L3 high
- Embryonic
- Increasing

Citations

1) Dineen, A., Osborne Nishimura, E., Goszczynski, B., Rothman, J. H., & McGhee, J. D. (2018). Quantitating transcription factor redundancy: The relative roles of the ELT-2 and ELT-7 GATA factors in the C. elegans endoderm. Developmental Biology, 435(2), 150–161. https://doi.org/10.1016/J.YDBIO.2017.12.023

2) Kudron, M. M., Victorsen, A., Gevirtzman, L., Hillier, L. W., Fisher, W. W., Vafeados, D., … Waterston, R. H. (2018). The modern resource: genome-wide binding profiles for hundreds of Drosophila and Caenorhabditis elegans transcription factors. Genetics, 208(3), 937–949. https://doi.org/10.1534/genetics.117.300657

Code

Source functions

```
source("./RWC23_Functions.R")
```

Load and Process Datasets

Load Dineen and Osborne Nishimura et. al. Data

```
dineen_nishimura_counts <-
  read_xlsx(path = "./01_input/Table_S2_rlog_Stabilized_Read_Counts.xlsx",
            sheet = "Sheet1")

dineen_nishimura_counts_matrix <- dineen_nishimura_counts %>%
  column_to_rownames(var = "WBGeneID")  %>%
  data.matrix()

dineen_nishimura_counts_matrix %>% head
```

```
##                wt_sorted_1 wt_sorted_2 wt_sorted_3 wt_sorted_4 elt7D_sorted_1
## WBGene00000001    8.957161    8.858238    8.841623    8.923111       8.505028
## WBGene00000002    7.489159    7.382905    7.518631    7.492399       7.378168
## WBGene00000003    9.061810    8.748589    9.295497    9.286834       9.480361
## WBGene00000004   10.916559   10.786200   11.010430   10.826657      10.836827
## WBGene00000005    2.990777    2.864044    3.116144    2.715502       2.584081
## WBGene00000007    5.799066    6.026780    5.831420    6.072836       5.699261
##                elt7D_sorted_2 elt7D_sorted_3 elt2D_sorted_1 elt2D_sorted_2
## WBGene00000001       8.568569       8.517438       9.172904       9.249496
## WBGene00000002       7.582425       7.512668       7.503760       7.289884
## WBGene00000003       9.451384       9.008938       8.669299       8.593847
## WBGene00000004      10.806534      10.819497      10.303062      10.296768
## WBGene00000005       2.881642       2.827526       2.953325       2.835451
## WBGene00000007       5.492677       5.220378       4.683237       4.797660
##                elt2D_sorted_3 elt2D_sorted_4 elt2Delt7D_sorted_1
## WBGene00000001       9.211660       9.346959            9.379698
## WBGene00000002       7.386127       7.262063            7.904008
## WBGene00000003       8.753835       8.781267            8.791018
## WBGene00000004      10.356820      10.366512           10.332489
## WBGene00000005       2.886842       2.979650            2.499412
## WBGene00000007       4.495252       4.593047            4.602235
```

```
##                 elt2Delt7D_sorted_2 elt2Delt7D_sorted_3
## WBGene00000001            9.217403            9.101997
## WBGene00000002            7.870852            7.762023
## WBGene00000003            8.795191            8.936724
## WBGene00000004           10.223675           10.597407
## WBGene00000005            2.763405            2.428255
## WBGene00000007            4.641832            4.476899
```

list of all dynamically expressed genes

```
dynamic_regulated_genes <-
  read.table(file = "./05_fromErin/2017-11-20_all_changing_genes_0.1alpha_0.8lfc.txt",
             quote = "",
             header = FALSE)
colnames(dynamic_regulated_genes) <- "WBGeneID"

dynamic_regulated_genes %>% head
```

```
##         WBGeneID
## 1 WBGene00004020
## 2 WBGene00015956
## 3 WBGene00000216
## 4 WBGene00001795
## 5 WBGene00008167
## 6 WBGene00010049
```

```
dineen_nishimura_clusters <-
  read_xlsx(path = "./01_input/Table_S6_All_Dynamically_Expressed_Genes_Clusters.xlsx",
            sheet = "dataset")

dineen_nishimura_sets <-
  dineen_nishimura_clusters %>% select(WBGeneID, set)
dineen_nishimura_sets_ascend <-
  arrange(dineen_nishimura_sets, WBGeneID)
dineen_nishimura_sets_ascend$set <-
  toupper(dineen_nishimura_sets_ascend$set)
dineen_nishimura_sets_ascend %>% head
```

```
## # A tibble: 6 x 2
##   WBGeneID       set
##   <chr>          <chr>
## 1 WBGene00000007 SET6
## 2 WBGene00000008 SET6
## 3 WBGene00000009 SET3
## 4 WBGene00000013 SET1
## 5 WBGene00000016 SET1
## 6 WBGene00000017 SET1
```

Load ELT-2 ChIP-seq binding annotations

```
elt2_peaks <-
  read_xlsx("./01_input/200410_peaksForBigBed.xlsx", sheet = "full cluster assignment")
```

```
## New names:
## * `` -> ...12
```

```
colnames(elt2_peaks) <-
  c(
    "chrom",
    "start",
    "end",
    "peak.name",
    "WBGeneID",
    "mapping",
    "cluster",
    "cluster.description",
    "kweight",
    "LE",
    "L1",
    "L3",
    "peak.summit.agreement"
  )

elt2_peaks$cluster.description <-
  factor(
    elt2_peaks$cluster.description,
    levels = c(
      "LE-specific",
      "Post-embryonic",
      "Increasing",
      "L3-high",
      "Not-changing or not IDR-passing"
    ),
    labels = c(
      "Embryo_Specific",
      "Larval",
      "Increasing",
      "L3_High",
      "Not_Changing"
    )
  )

elt2_cluster_names <- c("Embryo_Specific",
                        "Larval",
                        "Increasing",
                        "L3_High",
                        "Not_Changing")

elt2_peaks %>% head
```

```
## # A tibble: 6 x 13
##   chrom start   end peak.name WBGeneID mapping cluster cluster.descrip~ kweight
##   <chr> <dbl> <dbl> <chr>     <chr>    <chr>     <dbl> <fct>               <dbl>
## 1 chrI   3691  4222 ELT2peak~ WBGene0~ overla~       4 Increasing         0.818
## 2 chrI  11044 11533 ELT2peak~ WBGene0~ overla~       4 Increasing         0.913
## 3 chrI  13560 14890 ELT2peak~ WBGene0~ inside        2 Larval             0.876
## 4 chrI  15179 15647 ELT2peak~ WBGene0~ inside        4 Increasing         0.993
## 5 chrI  16706 17483 ELT2peak~ WBGene0~ overla~       3 L3_High            0.989
## 6 chrI  26789 27576 ELT2peak~ WBGene0~ downst~       1 Embryo_Specific    0.92
## # ... with 4 more variables: LE <dbl>, L1 <dbl>, L3 <dbl>,
```

```
## #   peak.summit.agreement <dbl>
```

Make a set of genes with ELT-2 binding detected in the L1 stage.

```
elt2_detected_in_L1 <-
  elt2_peaks %>% select(WBGeneID, L1) %>% filter(L1 == 1) %>% select(WBGeneID) %>% unique()

elt2_detected_in_L1 %>% head
```

```
## # A tibble: 6 x 1
##   WBGeneID
##   <chr>
## 1 WBGene00022277
## 2 WBGene00022276
## 3 WBGene00021026
## 4 WBGene00022038
## 5 WBGene00022043
## 6 WBGene00022042
```

```
elt2_detected_in_L1 %>% dim
```

```
## [1] 2430    1
```

Make a dataframe that records the number of peaks per gene that fall in a particular binding catagory.

```
binding_cluster_gene_counts <-
  table(elt2_peaks$WBGeneID, elt2_peaks$cluster.description)
binding_cluster_gene_counts <-
  as.data.frame.matrix(binding_cluster_gene_counts)
binding_cluster_gene_counts %>% head()
```

```
##                Embryo_Specific Larval Increasing L3_High Not_Changing
## WBGene00000003               0      0          1       0            0
## WBGene00000004               0      2          0       0            0
## WBGene00000007               0      0          1       0            0
## WBGene00000008               0      0          1       0            0
## WBGene00000009               0      1          1       0            0
## WBGene00000010               0      0          0       1            0
```

Load Spencer et. al. intestine expression

```
spencerLEgenes <-
  read.table(
    "../TF_Team/02_Data/6_Spencer_et_al_2010_FACS_and_pulldown_tilling_array/LE-intestine_enr_vs_ref.WS1
    quote = "\"",
    comment.char = "",
    header = TRUE
  )
colnames(spencerLEgenes) <-
  str_c("spencer_LE_", colnames(spencerLEgenes))
spencer_LE_subset <-
  spencerLEgenes %>% select(spencer_LE_ID,
                            spencer_LE_AveExpr,
                            spencer_LE_adj_P_Val,
                            spencer_LE_FC)

spencer_LE_subset %>% head
```

```
##    spencer_LE_ID spencer_LE_AveExpr spencer_LE_adj_P_Val spencer_LE_FC
## 1 WBGene00008163              7.57                    0         13.86
## 2 WBGene00021252              8.21                    0          7.30
## 3 WBGene00019986              9.29                    0         10.67
## 4 WBGene00007904              8.16                    0          6.89
## 5 WBGene00012018             10.14                    0          6.25
## 6 WBGene00010540              8.43                    0          4.15
```

```
spencerL2genes <-
  read.table(
    "../TF_Team/02_Data/6_Spencer_et_al_2010_FACS_and_pulldown_tilling_array/L2-intestine_enr_vs_ref.WS
    quote = "\"",
    comment.char = "",
    header = TRUE
  )
colnames(spencerL2genes) <-
  str_c("spencer_L2_", colnames(spencerL2genes))
spencer_L2_subset <- spencerL2genes %>%
  select(spencer_L2_ID,
         spencer_L2_AveExpr,
         spencer_L2_adj_P_Val,
         spencer_L2_FC)

spencer_L2_subset %>% head
```

```
##    spencer_L2_ID spencer_L2_AveExpr spencer_L2_adj_P_Val spencer_L2_FC
## 1 WBGene00020352              7.52                    0          7.51
## 2 WBGene00017225              7.28                    0          5.32
## 3 WBGene00007973              7.91                    0          5.93
## 4 WBGene00018683              8.27                    0          5.10
## 5 WBGene00003696              7.95                    0          3.73
## 6 WBGene00044776              7.77                    0          6.65
```

Process rlog counts

Subset rlog matrix based on presence in list `2017-11-20_all_changing_genes_0.1alpha_0.8lfc.txt`. Row scale and center the rlog counts per genes.

```
dynamic_counts_matrix <-
  matrix_select(dineen_nishimura_counts_matrix,
                dynamic_regulated_genes$WBGeneID)

dynamic_counts_matrix_scaled <-
  t(apply(unlist(dynamic_counts_matrix), 1, scale))

rownames(dynamic_counts_matrix_scaled) <-
  rownames(dynamic_counts_matrix)
colnames(dynamic_counts_matrix_scaled) <-
  colnames(dynamic_counts_matrix)
dynamic_counts_matrix_scaled %>% head
```

```
##                wt_sorted_1 wt_sorted_2 wt_sorted_3 wt_sorted_4 elt7D_sorted_1
## WBGene00000007   1.0068329  1.37348252   1.0589277   1.4476397     0.84613352
## WBGene00000008   2.2632093  1.13063525   1.1251278   1.0262925    -0.03607787
## WBGene00000009   0.1468716 -0.09556483  -0.3465276  -0.8378633     0.07003147
## WBGene00000013  -1.0765042  0.04628523  -1.0478603  -0.4296435    -0.61401384
```

```
## WBGene00000016  -0.1629274  0.14035593  -0.8318355  -0.2209018    -0.52814604
## WBGene00000017   0.1344074  0.43209491  -0.4453539   0.5202470    -0.19720767
##                elt7D_sorted_2 elt7D_sorted_3 elt2D_sorted_1 elt2D_sorted_2
## WBGene00000007     0.51350637     0.07506888     -0.7898010     -0.6055647
## WBGene00000008    -0.39030667     0.02722321     -0.4521136     -1.0292850
## WBGene00000009    -0.11586861     0.42221560      0.8406016      1.2349599
## WBGene00000013    -0.58009755    -0.38693983     -0.4767996      0.3851813
## WBGene00000016    -0.50445577    -0.16186256     -0.5681545     -0.6137809
## WBGene00000017     0.05519157     0.37152702     -0.9790560     -1.0378885
##                elt2D_sorted_3 elt2D_sorted_4 elt2Delt7D_sorted_1
## WBGene00000007    -1.09248186     -0.9350192          -0.9202246
## WBGene00000008    -0.46498937     -0.8771172          -0.9402531
## WBGene00000009     0.98161197      1.7266509          -1.7004545
## WBGene00000013     0.09286966     -0.5163112           2.5457794
## WBGene00000016    -0.75209134     -1.0136068           1.7015008
## WBGene00000017    -1.16996644     -1.7376299           1.4066491
##                elt2Delt7D_sorted_2 elt2Delt7D_sorted_3
## WBGene00000007          -0.8564679          -1.1220323
## WBGene00000008          -0.5550156          -0.8273297
## WBGene00000009          -0.8668929          -1.4597714
## WBGene00000013           1.4999051           0.5581492
## WBGene00000016           2.1353949           1.3805110
## WBGene00000017           1.6701858           0.9767996
```

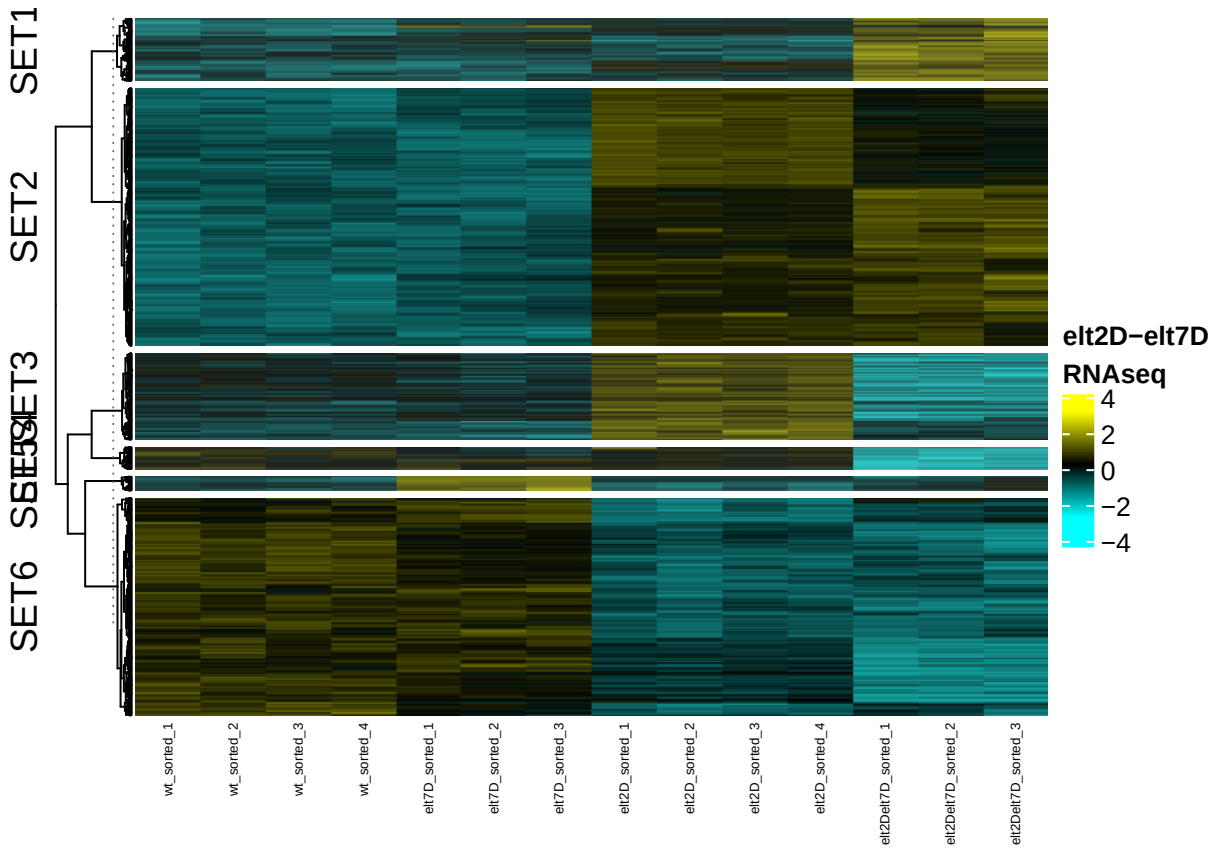
```
dynamic_counts_matrix_scaled_ascend <-
  dynamic_counts_matrix_scaled[order(rownames(dynamic_counts_matrix_scaled)),]
```

Must use arrange to sort genes in descending order to ensure row order is preserved

Recreate Supplementary Figure S4a from Dineen and Nishimura et al.

Use expression clusters from Dineen and Nishimura et al to split the clusters.

```
Heatmap(
  dynamic_counts_matrix_scaled_ascend,
  name = "elt2D-elt7D\nRNAseq",
  col = colorRampPalette(c("cyan", "black", "yellow"))(1000),
  cluster_columns = FALSE,
  clustering_distance_rows = "spearman",
  clustering_method_rows = "complete",
  show_row_names = FALSE,
  show_column_names = TRUE,
  row_names_gp = gpar(cex = 0.2),
  column_names_gp = gpar(cex = 0.4),
  heatmap_legend_param = list(color_bar = "continuous"),
  row_split = dineen_nishimura_sets_ascend$set
)
```

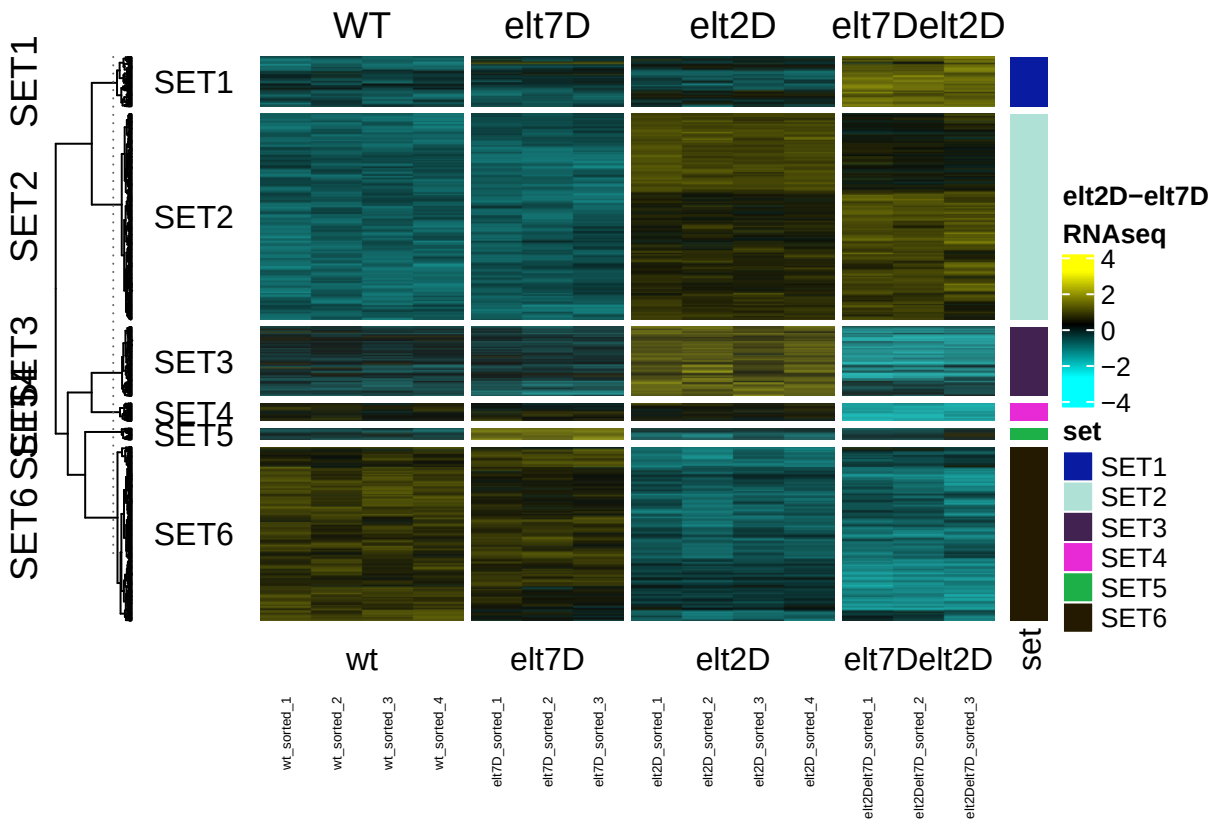



```
RNA_column_order <-
  factor(c(
    rep("WT", 4),
    rep("elt7D", 3),
    rep("elt2D", 4),
    rep("elt7Delt2D", 3)
  ),
  levels = c("WT", "elt7D", "elt2D", "elt7Delt2D"))
RNA_column_order
```

```
##  [1] WT          WT          WT          WT          elt7D       elt7D
##  [7] elt7D       elt2D       elt2D       elt2D       elt2D       elt7Delt2D
## [13] elt7Delt2D elt7Delt2D
## Levels: WT elt7D elt2D elt7Delt2D
```

```
Ha <- Heatmap(
  dynamic_counts_matrix_scaled_ascend,
  name = "elt2D-elt7D\nRNAseq",
  col = colorRampPalette(c("cyan", "black", "yellow"))(1000),
  cluster_columns = FALSE,
  clustering_distance_rows = "spearman",
  clustering_method_rows = "complete",
  show_row_names = FALSE,
  show_column_names = TRUE,
  row_names_gp = gpar(cex = 0.2),
  column_names_gp = gpar(cex = 0.4),
  heatmap_legend_param = list(color_bar = "continuous"),
  row_split = dineen_nishimura_sets_ascend$set,
```

```
  column_split = RNA_column_order,
  bottom_annotation = HeatmapAnnotation(foo = anno_block(
    labels = c("wt", "elt7D", "elt2D", "elt7Delt2D"),
    gp = gpar(border = NA, lty = "blank")
  )),
  left_annotation = rowAnnotation(foo = anno_block(
    labels = c("SET1", "SET2", "SET3", "SET4", "SET5", "SET6"),
    labels_rot = 0,
    gp = gpar(border = NA, lty = "blank")
  ))
)
Ha + rowAnnotation(set = dineen_nishimura_sets_ascend$set)
```



Add ELT-2 pattern row annotation

In ComplexHeatmap the row order of input matrix and annotation df must be identical to accurately plot data.

```
elt2_detected_in_L1 %>% dim
```

```
## [1] 2430    1
```

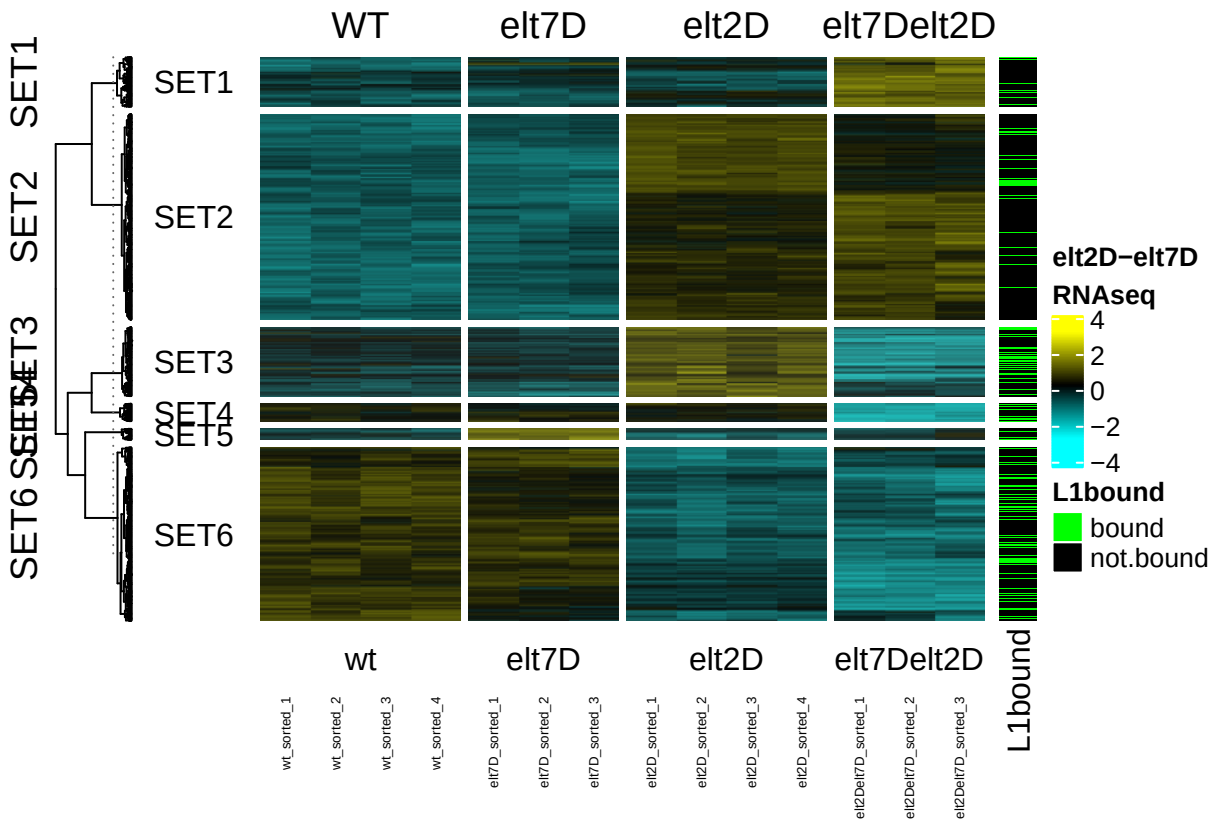
```
elt2_L1_anno <-
  data.frame(
    WBGeneID = rownames(dynamic_counts_matrix_scaled_ascend),
    elt2_detected_in_L1 = ifelse(
      test = rownames(dynamic_counts_matrix_scaled_ascend) %in% elt2_detected_in_L1$WBGeneID,
      yes = "bound",
      no = "not.bound"
```

```
    ),
    stringsAsFactors = FALSE
  )

elt2_L1_anno %>% head()
```

```
##         WBGeneID elt2_detected_in_L1
## 1 WBGene00000007           not.bound
## 2 WBGene00000008               bound
## 3 WBGene00000009           not.bound
## 4 WBGene00000013           not.bound
## 5 WBGene00000016           not.bound
## 6 WBGene00000017           not.bound
```

Incorporate this into a heatmap annotation

```
Ha_L1chip <-
  Ha + rowAnnotation(L1bound = elt2_L1_anno$elt2_detected_in_L1,
                     col = list(L1bound = c(
                       "bound" = "green", "not.bound" = "black"
                     )))

Ha_L1chip
```




```
# pdf("./03_plots/200428_wt_elt2singledouble_L1elt2bound_heatmap.pdf", height = 5, width = 5)
# Ha_L1chip
# dev.off()
```

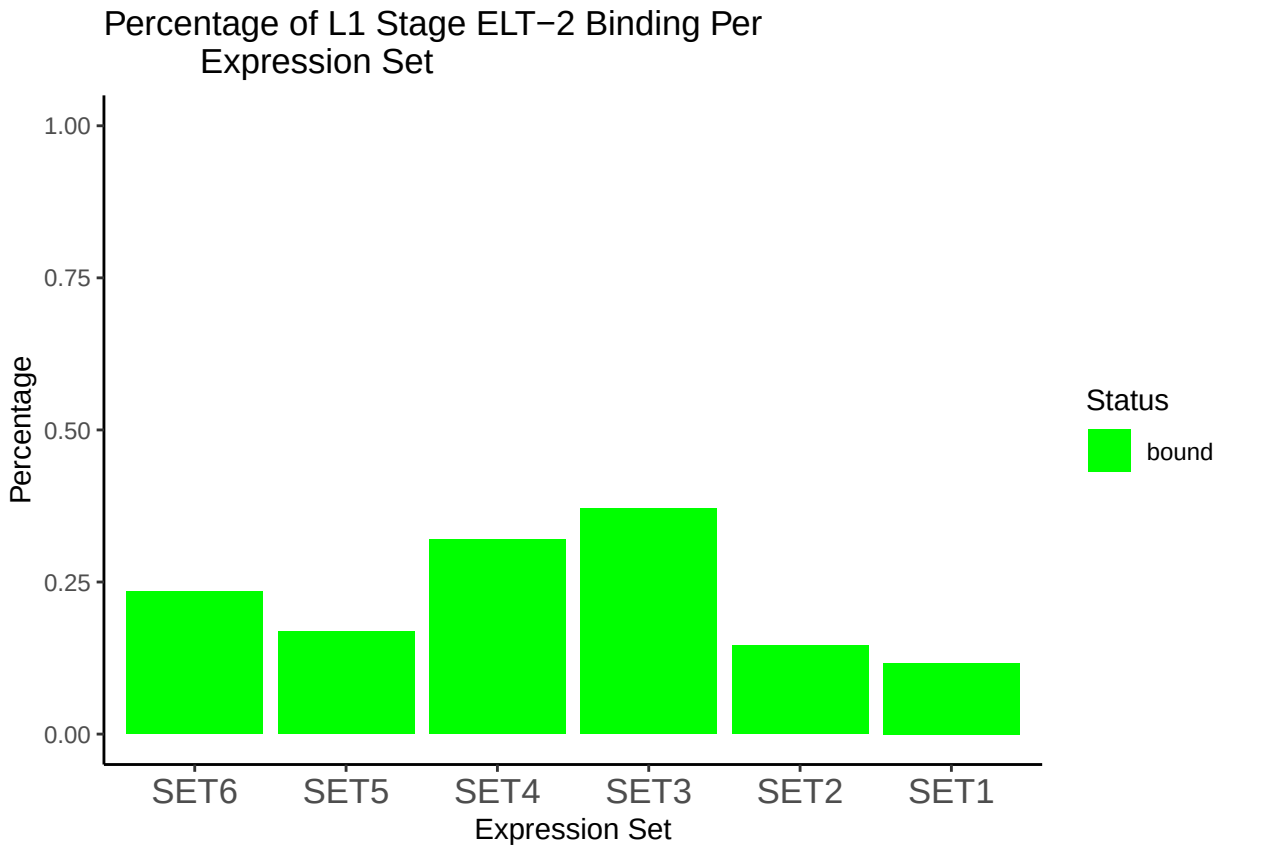
Determine enrichment of ELT-2 binding during L1 stage.

```r
expression_L1_binding <-
  merge(elt2_L1_anno, dineen_nishimura_sets_ascend, by = "WBGeneID")
expression_L1_binding %>% head
```

```
##         WBGeneID elt2_detected_in_L1  set
## 1 WBGene00000007           not.bound SET6
## 2 WBGene00000008               bound SET6
## 3 WBGene00000009           not.bound SET3
## 4 WBGene00000013           not.bound SET1
## 5 WBGene00000016           not.bound SET1
## 6 WBGene00000017           not.bound SET1
```

```r
clust_L1bound_counts <-
  table(expression_L1_binding$set,
        expression_L1_binding$elt2_detected_in_L1)

clust_L1bound_prop <- prop.table(clust_L1bound_counts, 1)

clust_L1bound_prop_ggplot <- as.data.frame(clust_L1bound_prop)

colnames(clust_L1bound_prop_ggplot) <- c("SET", "Status", "Freq")

clust_L1bound_prop_ggplot$Status <-
  factor(clust_L1bound_prop_ggplot$Status,
         levels = c("not.bound", "bound"))

clust_L1bound_prop_ggplot$SET <-
  factor(
    clust_L1bound_prop_ggplot$SET,
    levels = c("SET6", "SET5", "SET4", "SET3", "SET2", "SET1")
  )

clust_L1bound_colors <- c("bound" = "green", "not.bound" = "black")

l1bound_percents <-
  ggplot(
    clust_L1bound_prop_ggplot %>% filter(Status == "bound"),
    aes(
      x = SET,
      y = Freq,
      fill = Status,
      order = Status
    )
  ) +
  geom_bar(stat = "identity") +
  scale_color_manual(values = clust_L1bound_colors,
                     aesthetics = c("color", "fill")) +
  ggtitle("Percentage of L1 Stage ELT-2 Binding Per
          Expression Set") +
  xlab("Expression Set") +
  ylab("Percentage") +
  theme_classic() +
  theme(axis.text.x = element_text(size = 13)) +
  ylim(0, 1)
```

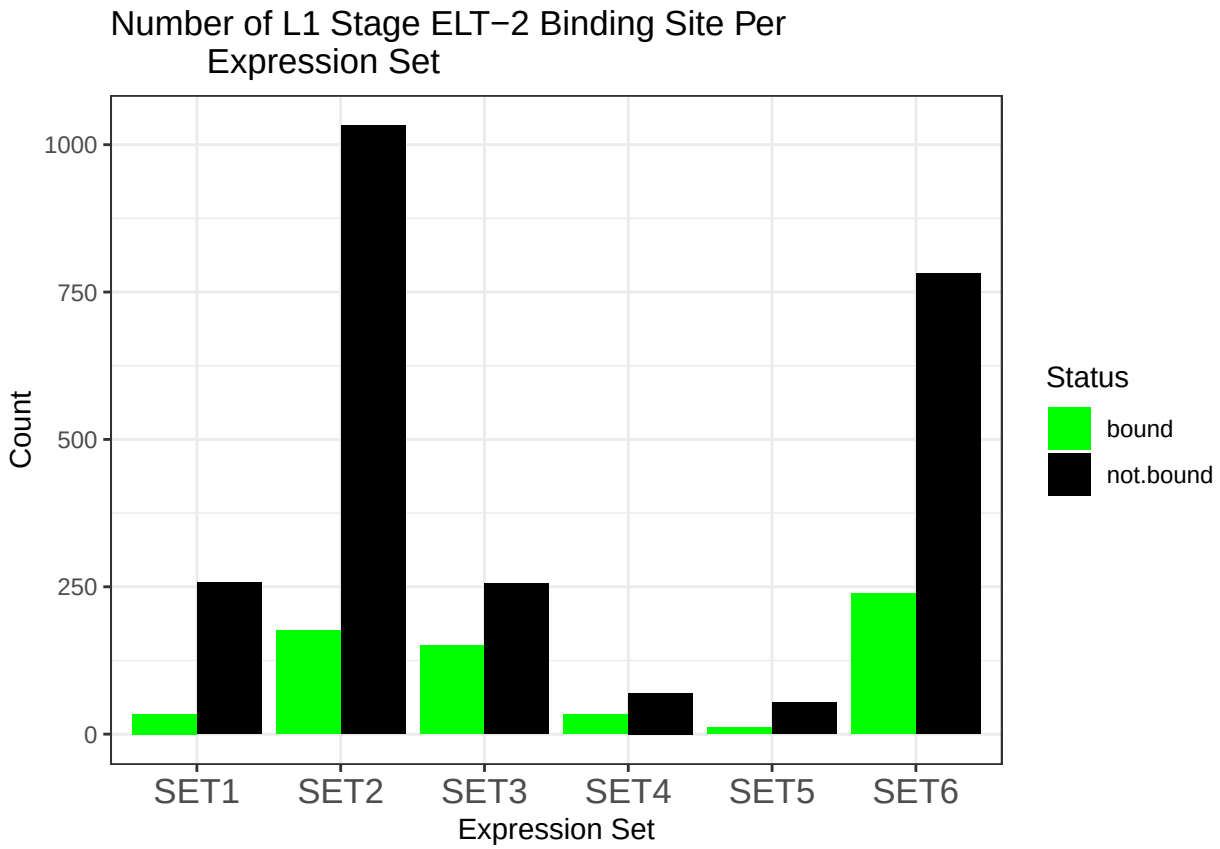
```
l1bound_percents
```




```
# ggsave("./03_plots/200428_proportion_of_l1elt2_per_expression_cluster.pdf", height = 2, width = 5)
```

Plot the number of "bound" vs "not.bound" per cluster

```
clust_L1bound_counts_ggplot <- as.data.frame(clust_L1bound_counts)
colnames(clust_L1bound_counts_ggplot) <- c("SET", "Status", "Freq")

ggplot(clust_L1bound_counts_ggplot,
       aes(x = SET,
           y = Freq,
           fill = Status)) +
  geom_bar(stat = "identity", position = "dodge") +
  scale_color_manual(values = clust_L1bound_colors,
                     aesthetics = c("color", "fill")) +
  ggtitle("Number of L1 Stage ELT-2 Binding Site Per
          Expression Set") +
  xlab("Expression Set") +
  ylab("Count") +
  theme_bw() +
  theme(axis.text.x = element_text(size = 13))
```




```
# ggsave("./03_plots/200428_number_of_l1elt2_per_expression_cluster.pdf", height = 2, width = 5)
```

Use the binomial test to determine if the different expression clusters are enriched or depleted for ELT-2 binding.

Use `binom.test` and first do a two-tailed test.

First calculate the proportion of bound genes over the total number of genes in the analysis.

```
proportion = as.numeric(colSums(clust_L1bound_counts)[1]) /
  as.numeric(colSums(clust_L1bound_counts)[1] + colSums(clust_L1bound_counts)[2])
proportion
```

```
## [1] 0.207956
```

Use custom function `ctable_binom()` to calculate p-vaule and confidence intervals for each set.

```
l1bound_binom <- ctable_binom(clust_L1bound_counts, "two.sided")
```

```
##    Set         pval conf.lower conf.upper  bool
## 1 SET1 6.426440e-05 0.08228607  0.1594291  TRUE
## 2 SET2 3.585965e-08 0.12626762  0.1668651  TRUE
## 3 SET3 8.109901e-14 0.32320354  0.4194467  TRUE
## 4 SET4 7.240238e-03 0.23184100  0.4195741  TRUE
## 5 SET5 5.413473e-01 0.08762605  0.2826562 FALSE
## 6 SET6 4.082629e-02 0.20862677  0.2615436  TRUE
```

This says that all sets but SET5 have a significant difference in genes bound compared to the entire dataset.

Now use the `less` or `greater` argument of `binom.test` to see if there is more or less binding.

First two `less`

```
ctable_binom(ctable = clust_L1bound_counts, alt = "less")
```

```
##    Set         pval conf.lower conf.upper  bool
## 1 SET1 3.238541e-05          0  0.1524937  TRUE
## 2 SET2 1.733956e-08          0  0.1634451  TRUE
## 3 SET3 1.000000e+00          0  0.4116901 FALSE
## 4 SET4 9.973903e-01          0  0.4041263 FALSE
## 5 SET5 2.752156e-01          0  0.2645358 FALSE
## 6 SET6 9.816208e-01          0  0.2571740 FALSE
```

This says that set 1 and 2 have less ELT-2 binding compared to the entire dataset.

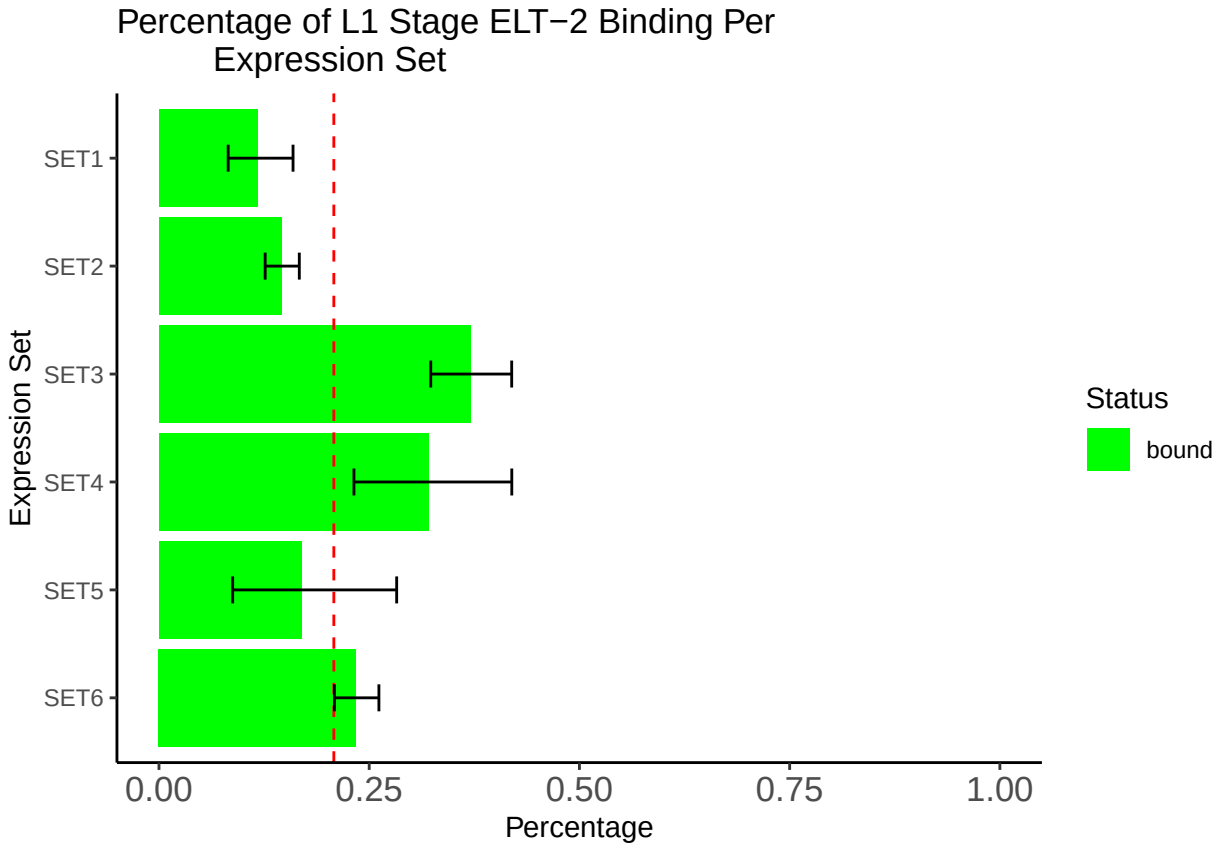
Now try `greater`.

```
ctable_binom(clust_L1bound_counts, "greater")
```

```
##    Set         pval conf.lower conf.upper  bool
## 1 SET1 9.999842e-01 0.08717538          1 FALSE
## 2 SET2 1.000000e+00 0.12923703          1 FALSE
## 3 SET3 4.779153e-14 0.33046830          1  TRUE
## 4 SET4 5.031706e-03 0.24460667          1  TRUE
## 5 SET5 8.207255e-01 0.09790359          1 FALSE
## 6 SET6 2.203126e-02 0.21259691          1  TRUE
```

Draw line on percentage plots to indicate background percentage of L1 binding.

```
l1bound_percents +
  geom_hline(yintercept = proportion,
             color = "red",
             linetype = "dashed") +
  geom_errorbar(
    ymax = l1bound_binom$conf.upper,
    ymin = l1bound_binom$conf.lower,
    width = 0.25
  ) +
  coord_flip()
```




```
# ggsave(
#   "./03_plots/200504_percentage_l1bound_per_expression_cluster.pdf",
#   width = 4,
#   height = 5
# )
```

Use the hypergeometric test to determine: Are changing genes (all sets) enriched for L1 binding?

```
N <- 20470
k <- nrow(elt2_detected_in_L1)
x3 <- as.numeric(colSums(clust_L1bound_counts)[1])
m <-
  as.numeric(colSums(clust_L1bound_counts)[1] + colSums(clust_L1bound_counts)[2])
dhyper(x3, m, N, k)
```

```
## [1] 1.05078e-78
```

Compute pairwise fisher's exact tests

```
fisher.multcomp(clust_L1bound_counts, p.method = "bonferroni")
```

```
##
##         Pairwise comparisons using Fisher's exact test for count data
##
## data:  clust_L1bound_counts
##
##           SET1      SET2      SET3   SET4 SET5
## SET2 1.000e+00         -         -      -    -
## SET3 2.072e-13 2.217e-19         -      -    -
```

```
## SET4 1.045e-04 2.783e-04 1.000e+00      -    -
## SET5 1.000e+00 1.000e+00 1.735e-02 0.4808    -
## SET6 9.200e-05 1.593e-06 5.232e-06 0.8164    1
##
## P value adjustment method: bonferroni
```

```
fisher.multcomp(clust_L1bound_counts, p.method = "bonferroni")$p.value < 0.05
```

```
##       SET1  SET2  SET3  SET4  SET5
## SET2 FALSE    NA    NA    NA    NA
## SET3  TRUE  TRUE    NA    NA    NA
## SET4  TRUE  TRUE FALSE    NA    NA
## SET5 FALSE FALSE  TRUE FALSE    NA
## SET6  TRUE  TRUE  TRUE FALSE FALSE
```

Row annotation of ELT-2 Binding Pattern Clusters

```
dynamic_counts_matrix_scaled %>% dim
```

```
## [1] 3092   14
```

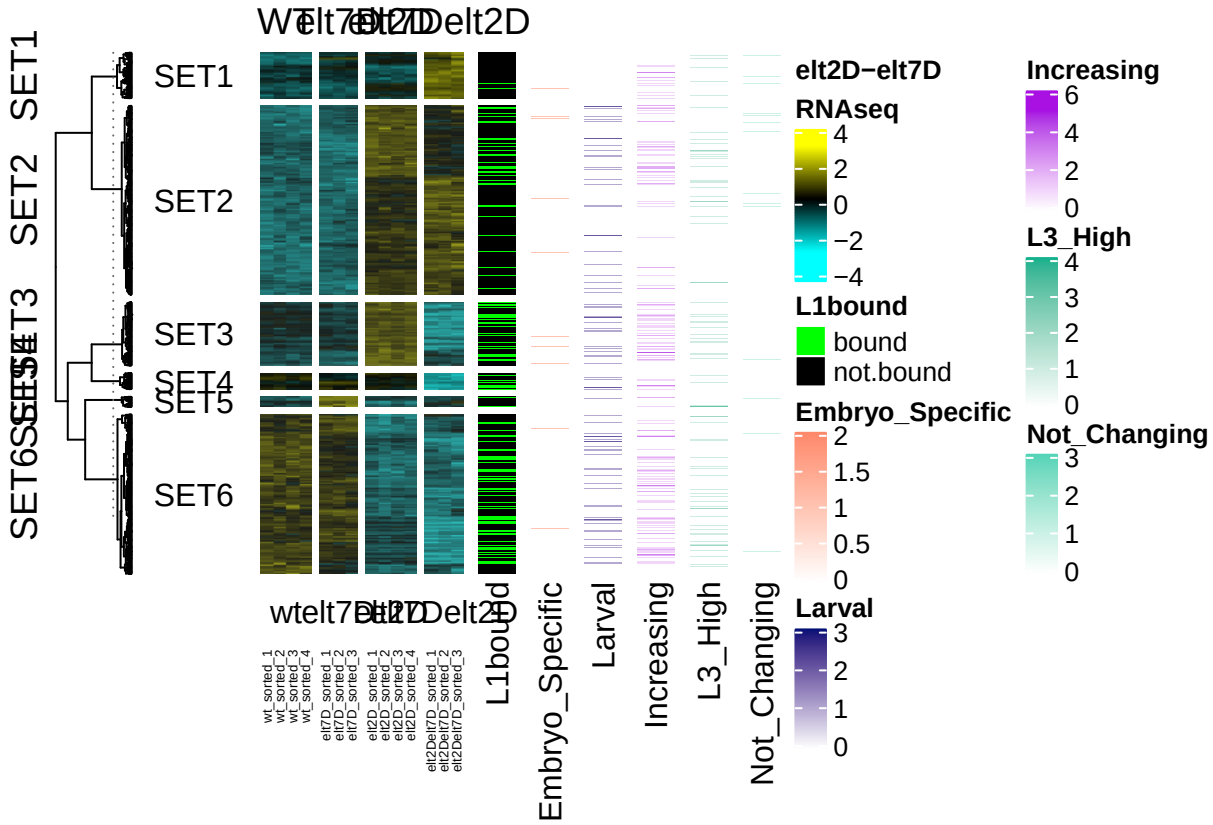
```
chip_annotation <-
  make_cluster_annotation(dynamic_counts_matrix_scaled_ascend,
                          binding_cluster_gene_counts)

unique(rownames(dynamic_counts_matrix_scaled_ascend) == chip_annotation$rowname)
```

```
## [1] TRUE
```

```
nrow(dynamic_counts_matrix_scaled) == nrow(chip_annotation)
```

```
## [1] TRUE
```

```
Ha_L1chip_bindcluster <- Ha_L1chip +
  rowAnnotation(Embryo_Specific = chip_annotation$Embryo_Specific) +
  rowAnnotation(Larval = chip_annotation$Larval) +
  rowAnnotation(Increasing = chip_annotation$Increasing) +
  rowAnnotation(L3_High = chip_annotation$L3_High) +
  rowAnnotation(Not_Changing = chip_annotation$Not_Changing)
Ha_L1chip_bindcluster
```



Have the colors match plot from David.

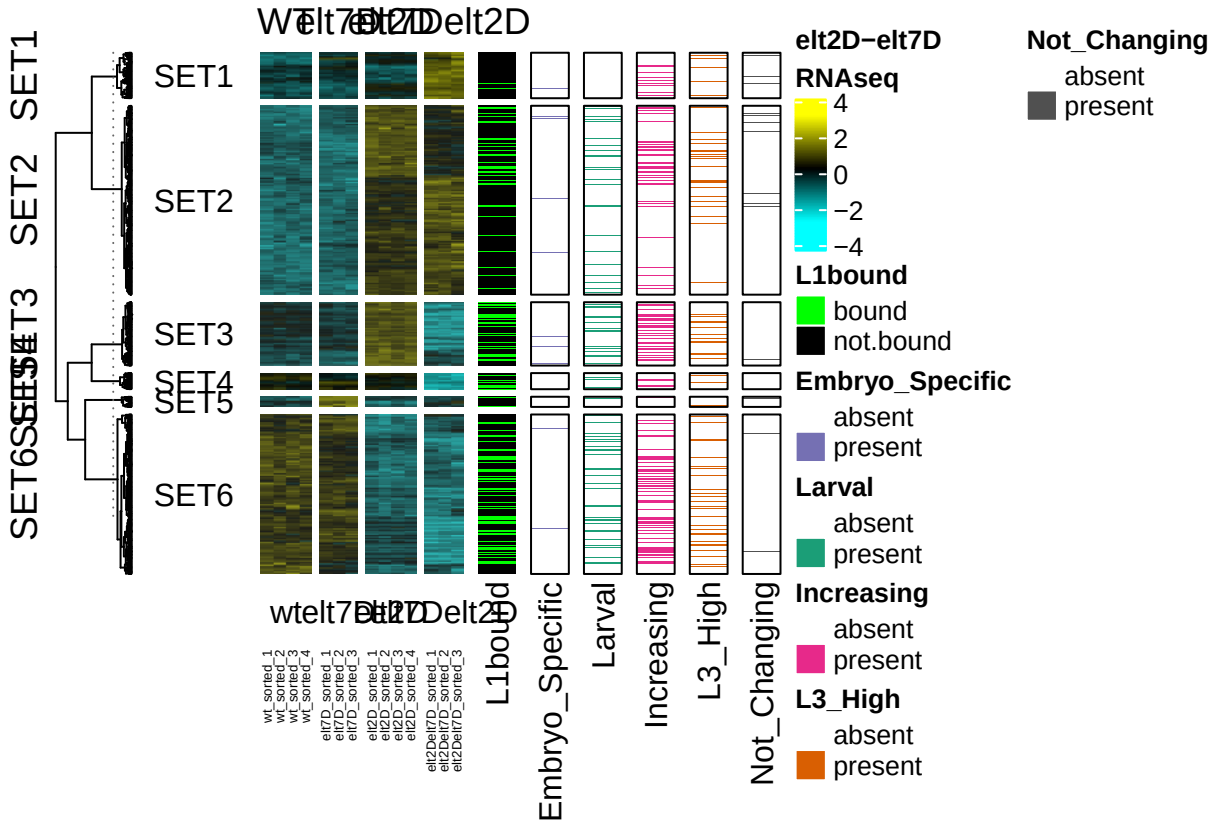
```
cluster_colors <-
  data.frame(
    class = elt2_cluster_names,
    val = c("#7570B3", "#1B9E77", "#E7298A", "#D95F02", "#505050")
  )

cluster_colors$class <-
  factor(x = cluster_colors$class,
         levels = elt2_cluster_names)
```

Convert ChIP binding clusters to a present/absence list.

```
chip_annotation_present_absent <-
  make_cluster_binary_annotation(chip_annotation)
```

Plot the heatmap with presence/absence.

```
Ha_L1chip_clusterchip <-
  Ha_L1chip + binding_cluster_row_annotation(chip_annotation_present_absent)

Ha_L1chip_clusterchip
```




```
# pdf("./03_plots/200504_wt_elt2singledouble_L1elt2bound_elt2bindclusters_heatmap.pdf", height = 5, wid
# Ha_L1chip_clusterchip
# dev.off()
```

Plot percentage of expression cluster group having binding pattern assignment.

```
exprclust_bindclust <-
  merge(
    dineen_nishimura_sets_ascend,
    chip_annotation_present_absent,
    by.x = "WBGeneID",
    by.y = "rowname"
  )

exprclust_bindclust %>% head
```

```
##         WBGeneID  set Embryo_Specific  Larval Increasing L3_High Not_Changing
## 1 WBGene00000007 SET6          absent  absent    present  absent       absent
## 2 WBGene00000008 SET6          absent  absent    present  absent       absent
## 3 WBGene00000009 SET3          absent present    present  absent       absent
## 4 WBGene00000013 SET1          absent  absent     absent  absent       absent
## 5 WBGene00000016 SET1          absent  absent     absent  absent       absent
## 6 WBGene00000017 SET1          absent  absent     absent  absent       absent
```

What is the percentage of genes with annotated ELT2 binding clusters per expression dataset?

Make a dataframe that addresses the question:

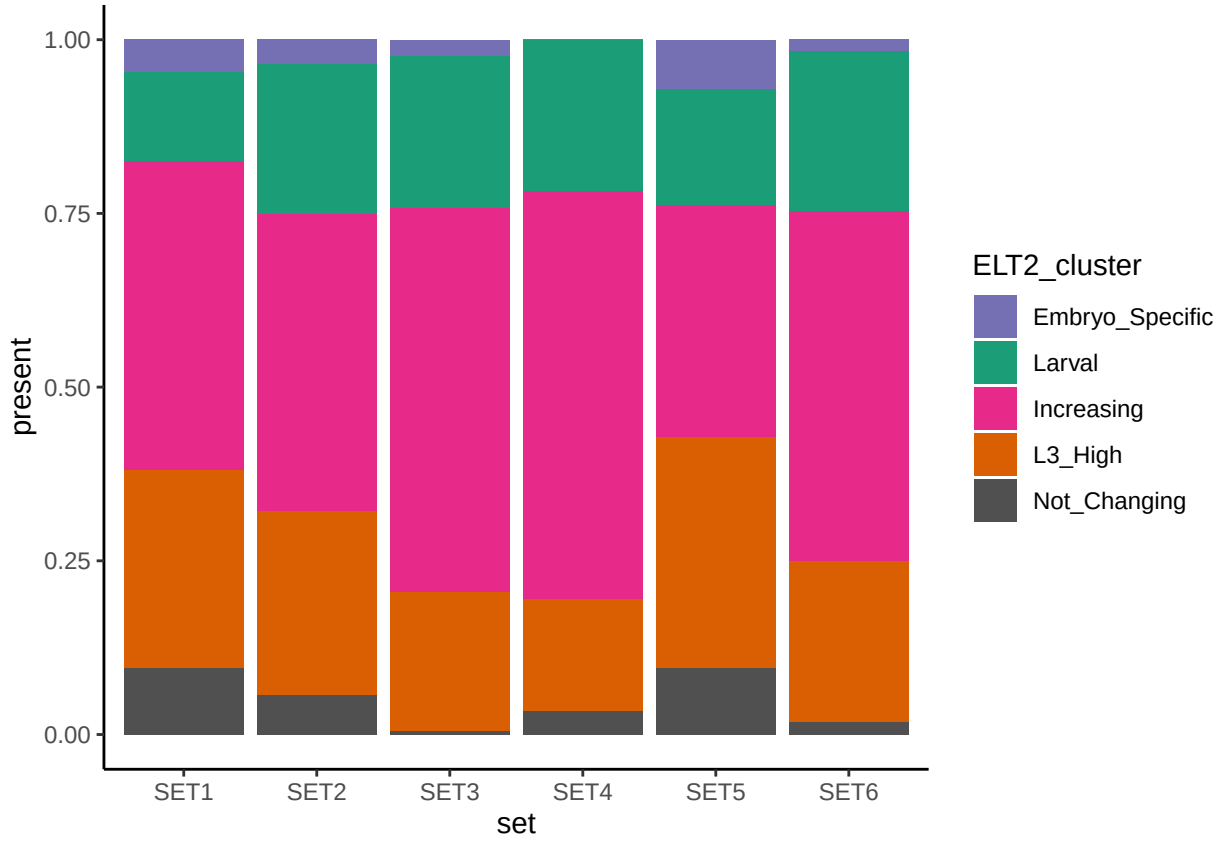
```
expressionSet_per_BindingCluster <- data.frame()
for (i in elt2_cluster_names) {
  toappend <-
    table(exprclust_bindclust$set,
          exprclust_bindclust[[i]]) %>%
    as.data.frame.matrix() %>%
    rownames_to_column(var = "set") %>%
    mutate(ELT2_cluster = i,
           percent = present / (present + absent))
  expressionSet_per_BindingCluster <-
    bind_rows(expressionSet_per_BindingCluster, toappend)
}

expressionSet_per_BindingCluster$ELT2_cluster <-
  factor(expressionSet_per_BindingCluster$ELT2_cluster, levels = elt2_cluster_names)

expressionSet_per_BindingCluster
```

```
##     set absent present    ELT2_cluster     percent
## 1  SET1    285       6 Embryo_Specific 0.020618557
## 2  SET2   1187      21 Embryo_Specific 0.017384106
## 3  SET3    397       8 Embryo_Specific 0.019753086
## 4  SET4    103       0 Embryo_Specific 0.000000000
## 5  SET5     62       3 Embryo_Specific 0.046153846
## 6  SET6   1009      11 Embryo_Specific 0.010784314
## 7  SET1    275      16          Larval 0.054982818
## 8  SET2   1077     131          Larval 0.108443709
## 9  SET3    328      77          Larval 0.190123457
## 10 SET4     84      19          Larval 0.184466019
## 11 SET5     58       7          Larval 0.107692308
## 12 SET6    874     146          Larval 0.143137255
## 13 SET1    235      56      Increasing 0.192439863
## 14 SET2    950     258      Increasing 0.213576159
## 15 SET3    212     193      Increasing 0.476543210
## 16 SET4     52      51      Increasing 0.495145631
## 17 SET5     51      14      Increasing 0.215384615
## 18 SET6    700     320      Increasing 0.313725490
## 19 SET1    255      36         L3_High 0.123711340
## 20 SET2   1048     160         L3_High 0.132450331
## 21 SET3    335      70         L3_High 0.172839506
## 22 SET4     89      14         L3_High 0.135922330
## 23 SET5     51      14         L3_High 0.215384615
## 24 SET6    872     148         L3_High 0.145098039
## 25 SET1    279      12    Not_Changing 0.041237113
## 26 SET2   1174      34    Not_Changing 0.028145695
## 27 SET3    403       2    Not_Changing 0.004938272
## 28 SET4    100       3    Not_Changing 0.029126214
## 29 SET5     61       4    Not_Changing 0.061538462
## 30 SET6   1009      11    Not_Changing 0.010784314
```

Make a plot that addresses the question:

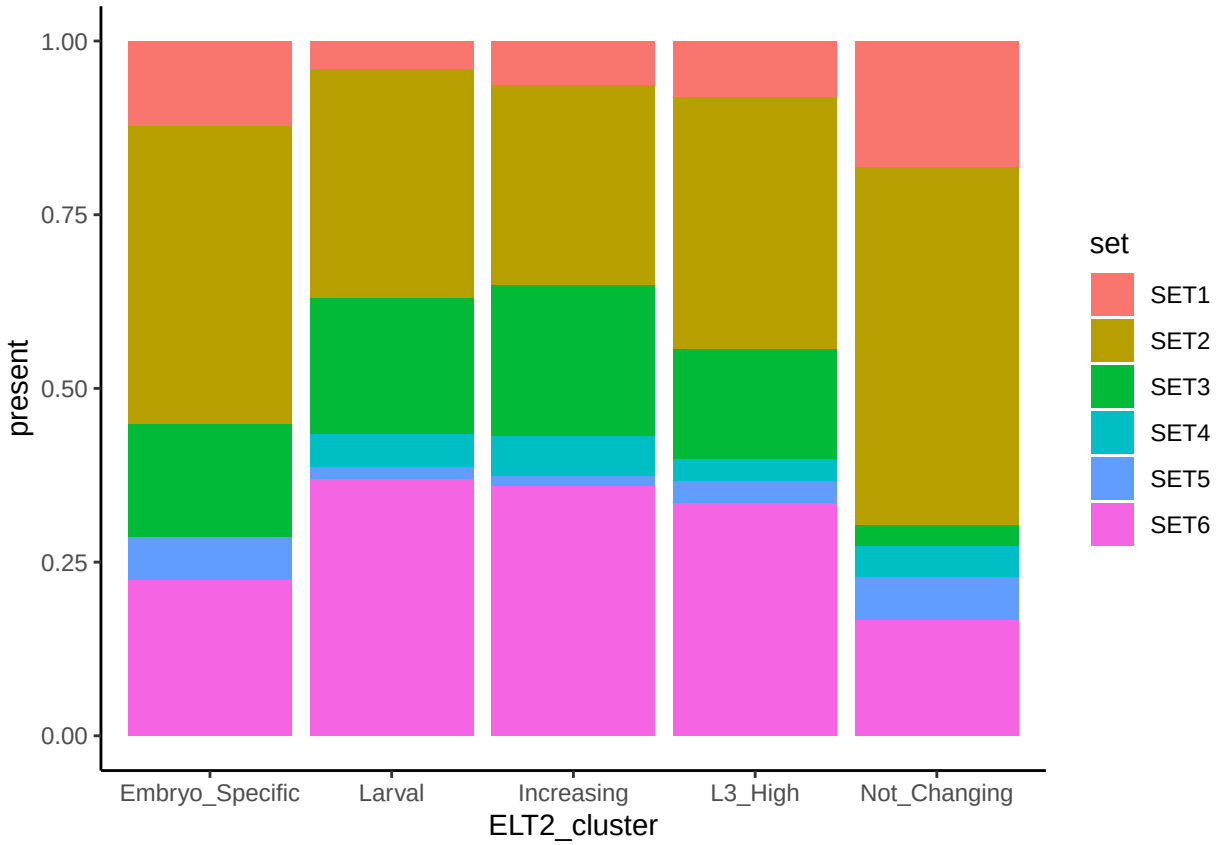
```
ggplot(expressionSet_per_BindingCluster,
       aes(x = set,
           y = present,
```

```
          fill = ELT2_cluster)) +
  geom_bar(stat = "identity", position = "fill") +
  theme_classic() +
  scale_fill_manual(values = as.vector(cluster_colors$val))
```



What is the percentage of genes within each Expression Set that are associated with an ELT-2 binding cluster?

```
ggplot(expressionSet_per_BindingCluster,
       aes(x = ELT2_cluster, y = present, fill = set)) +
  geom_bar(stat = "identity", position = "fill") +
  theme_classic()
```



Make a series of horizontal barplots with percentage of ELT-2 binding cluster per expression cluster.

TODO: Make a function that does all that is below.

```
setStats <- function(){

}
```

First, calculate the percentage of each ELT-2 binding category against the total dataset.

```
percent_bound_per_ELT2_cluster <-
  expressionSet_per_BindingCluster %>% group_by(ELT2_cluster) %>% summarise(percent = sum(present) /
                                                                              nrow(dynamic_counts_matri
```

Next calculate the the 95% Confidence Interval with the Bionomial Test.

```
expressionSet_per_BindingCluster %>% group_by(set, ELT2_cluster) %>% summarise(percent = present /
                                                                                 (present + absent))
```

```
## # A tibble: 30 x 3
## # Groups:   set [6]
##    set   ELT2_cluster    percent
##    <chr> <fct>              <dbl>
##  1 SET1  Embryo_Specific  0.0206
##  2 SET1  Larval           0.0550
##  3 SET1  Increasing       0.192
##  4 SET1  L3_High          0.124
##  5 SET1  Not_Changing     0.0412
##  6 SET2  Embryo_Specific  0.0174
##  7 SET2  Larval           0.108
```

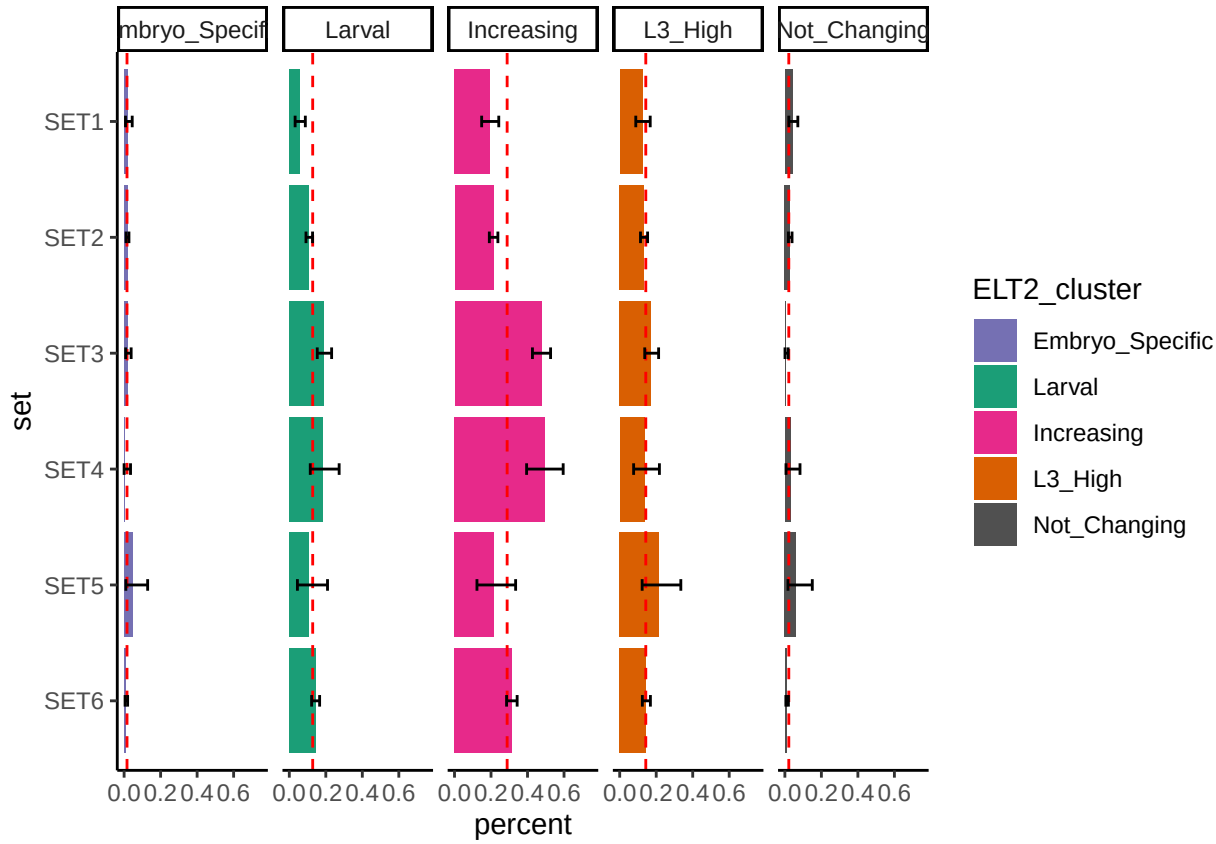
```
##  8 SET2  Increasing       0.214
##  9 SET2  L3_High          0.132
## 10 SET2  Not_Changing     0.0281
## # ... with 20 more rows
```

Calculate the binomial pvalue and confidence intervals.

```
# Add a column for the background percentage of ELT2 binding clusters per the whole expression dataset
expression_binding_stats <-
  expressionSet_per_BindingCluster %>% group_by(ELT2_cluster) %>% mutate(background_percent = sum(prese
                                                                                                  (sum(present) + sum(absent))

# Use binom.test to calculate pvalue and confidence intervales for the percentage of ELT2 binding clust
expression_binding_stats <- expression_binding_stats %>%
  group_by(ELT2_cluster, set) %>%
  mutate(
    pval = binom.test(
      x = c(present, absent),
      n = present + absent,
      p = background_percent,
      alternative = "two.sided"
    )$p.value,
    conf.upper = binom.test(
      x = c(present, absent),
      n = present + absent,
      p = background_percent,
      alternative = "two.sided"
    )$conf.int[2],
    conf.lower = binom.test(
      x = c(present, absent),
      n = present + absent,
      p = background_percent,
      alternative = "two.sided"
    )$conf.int[1]
  )

expression_binding_stats$set <-
  factor(
    expression_binding_stats$set,
    levels = c("SET6", "SET5", "SET4", "SET3", "SET2", "SET1")
  )

expression_binding_stats %>% head()
```

```
## # A tibble: 6 x 9
## # Groups:   ELT2_cluster, set [6]
##   set   absent present ELT2_cluster percent background_perc~   pval conf.upper
##   <fct>  <int>   <int> <fct>          <dbl>            <dbl>  <dbl>      <dbl>
## 1 SET1     285       6 Embryo_Spec~  0.0206           0.0158 0.475      0.0443
## 2 SET2    1187      21 Embryo_Spec~  0.0174           0.0158 0.644      0.0265
## 3 SET3     397       8 Embryo_Spec~  0.0198           0.0158 0.545      0.0385
## 4 SET4     103       0 Embryo_Spec~  0                0.0158 0.417      0.0352
## 5 SET5      62       3 Embryo_Spec~  0.0462           0.0158 0.0844     0.129
## 6 SET6    1009      11 Embryo_Spec~  0.0108           0.0158 0.257      0.0192
## # ... with 1 more variable: conf.lower <dbl>
```

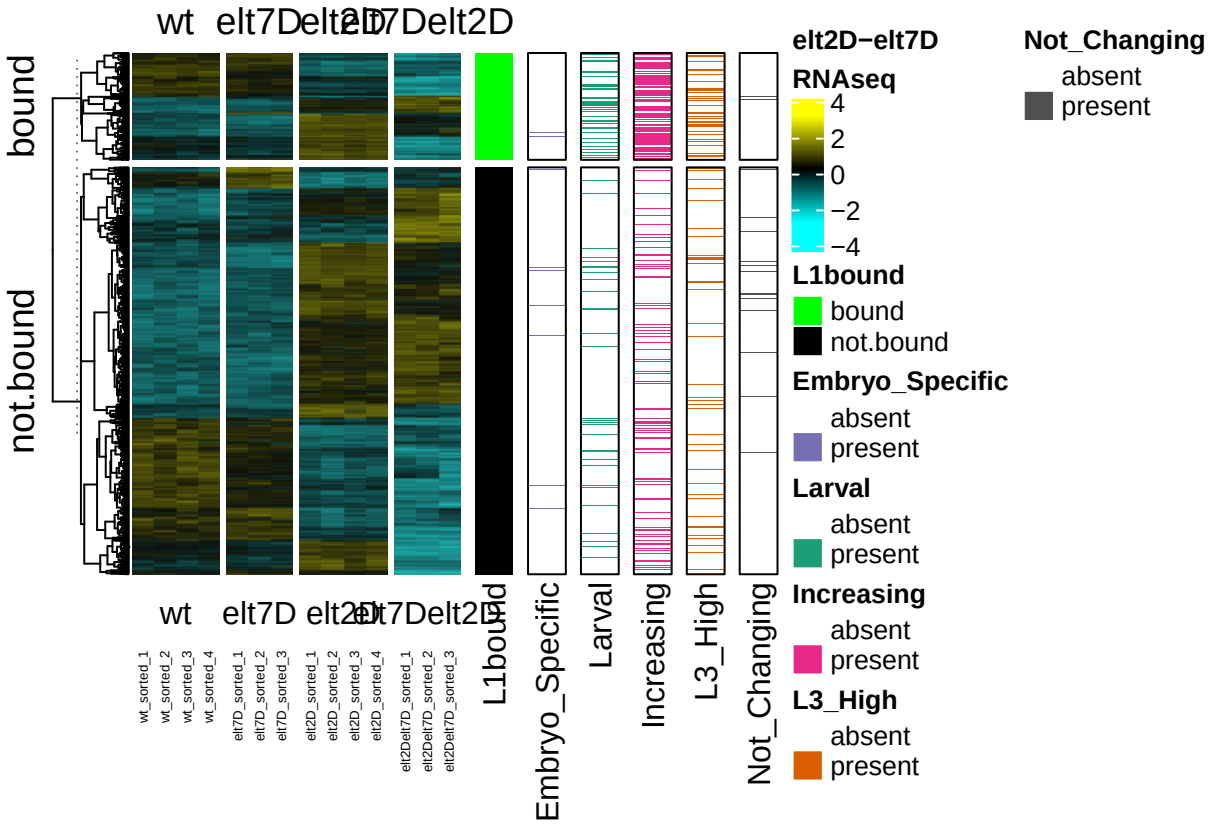
```
ggplot(expression_binding_stats,
       aes(x = set,
           y = percent, fill = ELT2_cluster)) +
  geom_bar(stat = "identity") +
  scale_y_continuous(limits = c(0, 0.75)) +
  theme_classic() +
  geom_hline(
    data = percent_bound_per_ELT2_cluster,
    color = "red",
    linetype = "dashed",
    aes(yintercept = percent)
  ) +
  geom_errorbar(
    ymax = expression_binding_stats$conf.upper,
    ymin = expression_binding_stats$conf.lower,
    width = 0.1
  ) +
  coord_flip() +
  facet_grid(. ~ ELT2_cluster) +
  scale_fill_manual(values = as.character(cluster_colors$val))
```




```
# ggsave(filename = "./03_plots/200511_Percent_of_ELT2bindClust_per_ExpressionClust.pdf")
```

Subset ELT-2/ELT-7 differentially expressed genes based on ELT-2 binding in L1 stage

```
RNA_heatmap(dynamic_counts_matrix_scaled_ascend,
            split = elt2_L1_anno$elt2_detected_in_L1) +
  elt2_l1_row_annotation(elt2_L1_anno) +
  binding_cluster_row_annotation(chip_annotation_present_absent)
```




```
l1_bound_list <-
  elt2_L1_anno %>% filter(elt2_detected_in_L1 == "bound") %>% select(WBGeneID) %>% arrange(WBGeneID)

dynamic_counts_matrix_scaled_bound_only <-
  matrix_select(dynamic_counts_matrix_scaled_ascend, l1_bound_list$WBGeneID)

bound_only_elt2_clust_anno <-
  make_cluster_binary_annotation(
    make_cluster_annotation(
      dynamic_counts_matrix_scaled_bound_only,
      binding_cluster_gene_counts
    )
  )

bound_only_elt2_clust_anno %>% head()
```

```
##          rowname Embryo_Specific  Larval Increasing L3_High Not_Changing
## 1 WBGene00000008          absent  absent    present  absent       absent
## 2 WBGene00000064          absent  absent    present present       absent
```

```
## 3 WBGene00000067          absent present    present  absent       absent
## 4 WBGene00000107          absent  absent    present  absent       absent
## 5 WBGene00000136          absent present    present  absent       absent
## 6 WBGene00000172          absent  absent    present  absent       absent
```

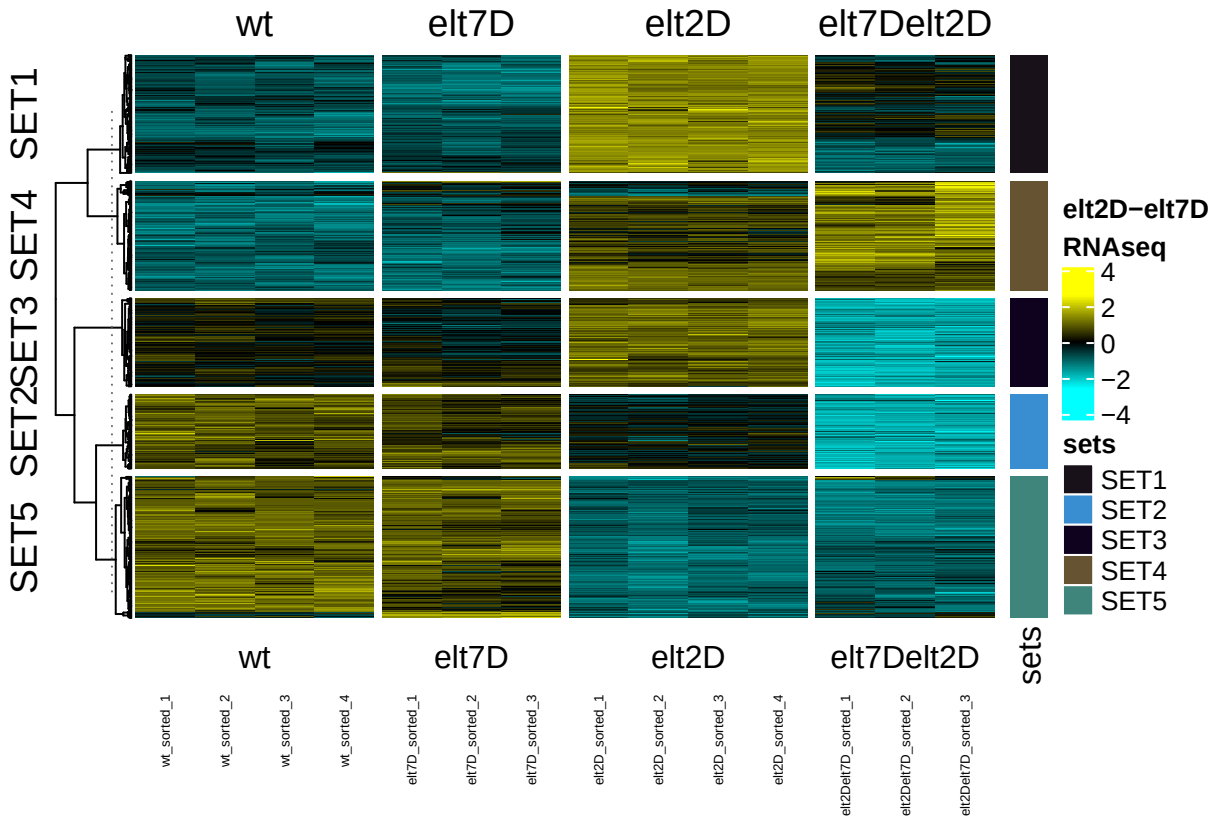
Assign k-means clusters for rows before plotting

```
kclus <- kmeans(dynamic_counts_matrix_scaled_bound_only, 5)
bound_only_sets <-
  data.frame(
    WBGeneID = rownames(dynamic_counts_matrix_scaled_bound_only),
    set = paste("SET", kclus$cluster, sep = "")
  )
head(bound_only_sets)
```

```
##         WBGeneID  set
## 1 WBGene00000008 SET5
## 2 WBGene00000064 SET4
## 3 WBGene00000067 SET1
## 4 WBGene00000107 SET5
## 5 WBGene00000136 SET2
## 6 WBGene00000172 SET3
```

Draw heatmap and check that set assignment is correct.

```
Ha_bound_only <-
  RNA_heatmap(dynamic_counts_matrix_scaled_bound_only,
              bound_only_sets$set) +
  rowAnnotation(sets = bound_only_sets$set)

Ha_bound_only
```

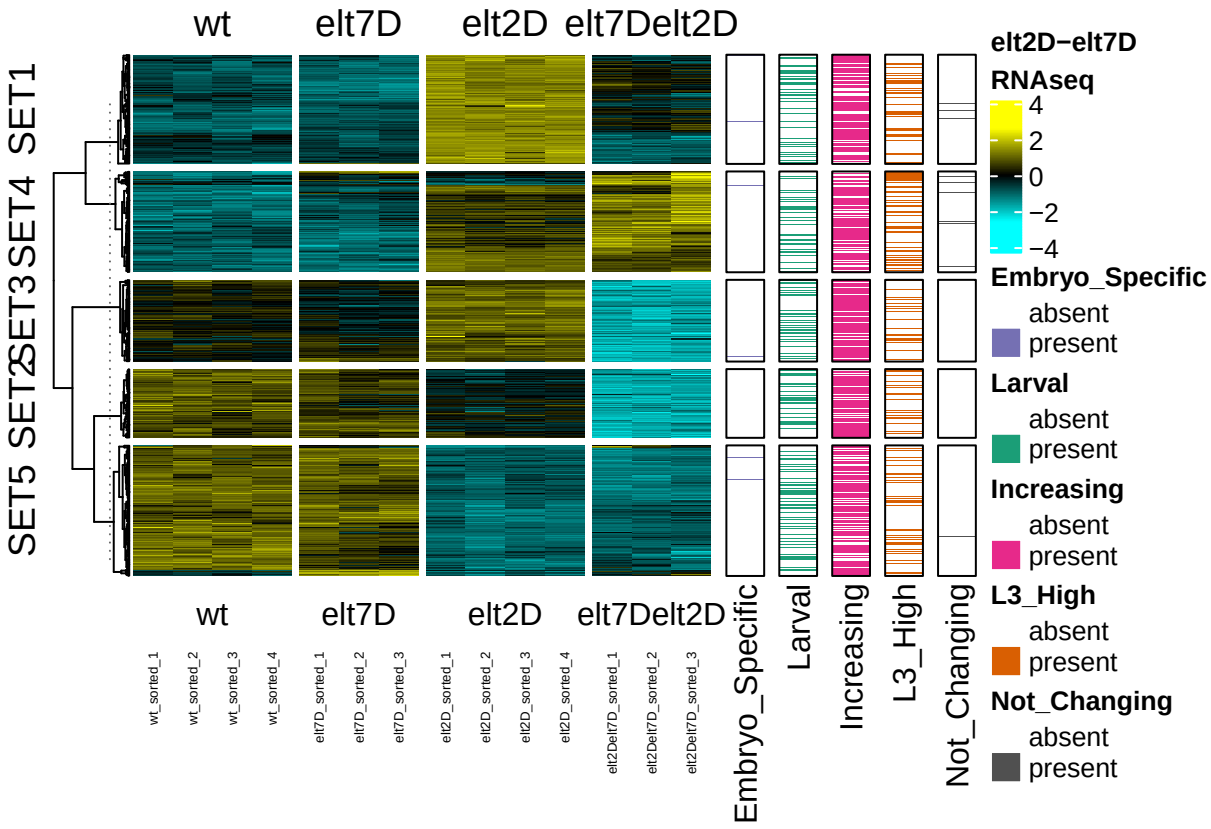



```
Ha_bound_only <-
  RNA_heatmap(dynamic_counts_matrix_scaled_bound_only,
              bound_only_sets$set)
```

```
bound_only_annotation <-
  merge(bound_only_elt2_clust_anno,
        bound_only_sets,
        by.x = "rowname",
        by.y = "WBGeneID")
bound_only_annotation_ascend <-
  bound_only_annotation %>% arrange(rowname)
head(bound_only_annotation_ascend)
```

```
##          rowname Embryo_Specific  Larval Increasing L3_High Not_Changing  set
## 1 WBGene00000008          absent  absent    present  absent       absent SET5
## 2 WBGene00000064          absent  absent    present present       absent SET4
## 3 WBGene00000067          absent present    present  absent       absent SET1
## 4 WBGene00000107          absent  absent    present  absent       absent SET5
## 5 WBGene00000136          absent present    present  absent       absent SET2
## 6 WBGene00000172          absent  absent    present  absent       absent SET3
```

```
Ha_bound_only_chipClust <-
  Ha_bound_only + binding_cluster_row_annotation(bound_only_elt2_clust_anno)
Ha_bound_only_chipClust
```



L1 Intestine expression row annotation

Import RWC24 L1 Intestine results table

```
RWC24_res <-
  read.csv(
    "../RWC24_L1_Intestine_RNAseq/04_DEseq2/200511_L1_intestine_FACS_gut_vs_gutless.csv",
    row.names = 1
  )
RWC24_res <- RWC24_res %>% rownames_to_column(var = "WBGeneID")
head(RWC24_res)
```

```
##         WBGeneID baseMean log2FoldChange lfcSE stat pvalue padj
## 1 WBGene00014451        0             NA    NA   NA     NA   NA
## 2 WBGene00010957        0             NA    NA   NA     NA   NA
## 3 WBGene00010958        0             NA    NA   NA     NA   NA
## 4 WBGene00014452        0             NA    NA   NA     NA   NA
## 5 WBGene00014453        0             NA    NA   NA     NA   NA
## 6 WBGene00014454        0             NA    NA   NA     NA   NA
```

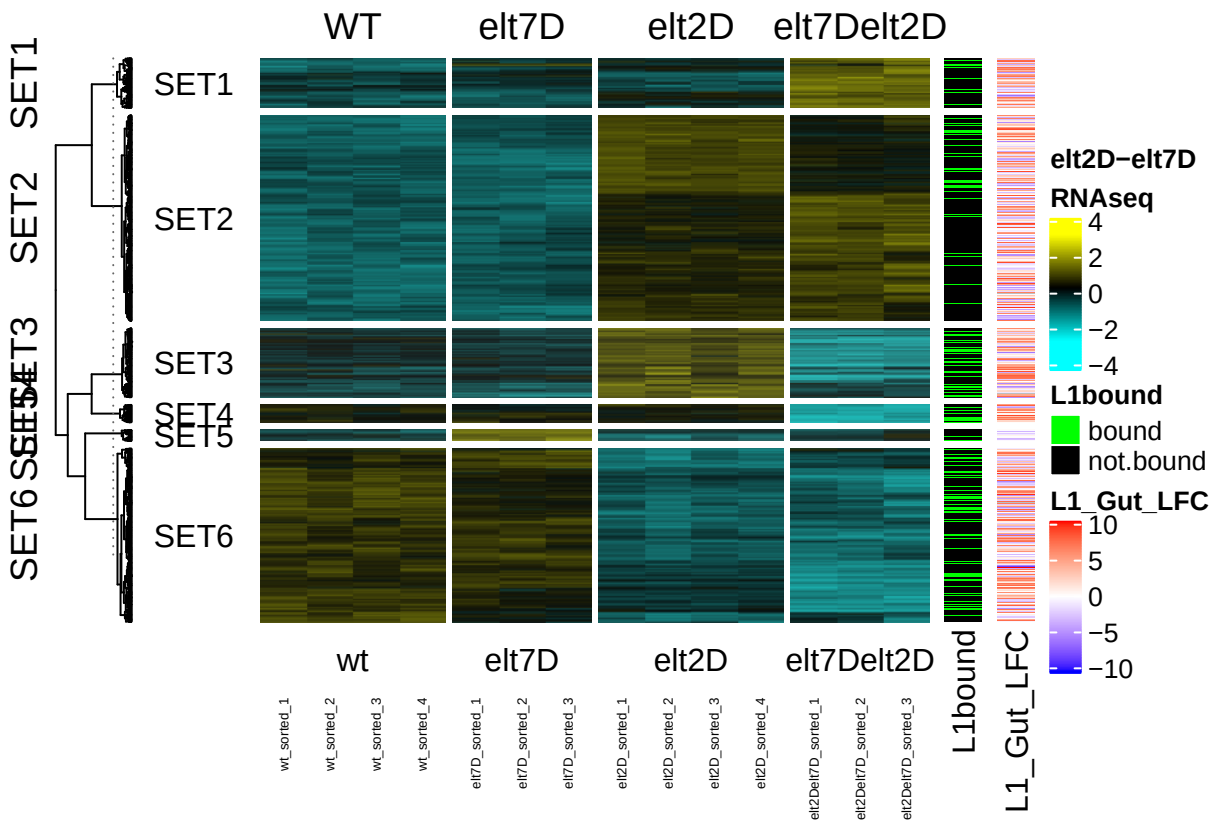
Select log fold change data for row annotation.

```
L1_gut_lfc <-
  dynamic_counts_matrix_scaled_ascend %>% as.data.frame.matrix() %>% rownames_to_column(var = "WBGeneID
```

Add to heatmap as a row annotation.

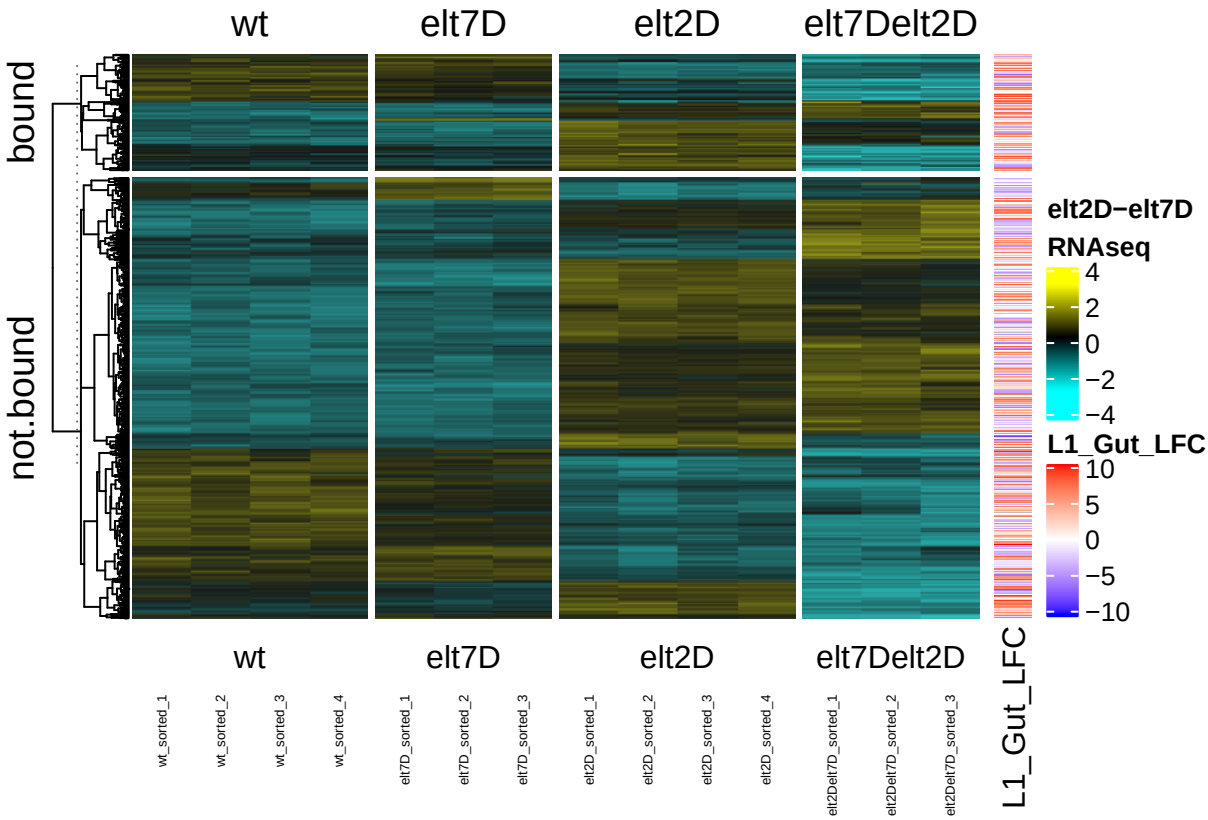
```
col_fun = colorRamp2(c(-10, 0, 10), c("blue", "white", "red"))
```

```
Ha_L1chip_L1gutLFC <-
  Ha_L1chip + rowAnnotation(L1_Gut_LFC = L1_gut_lfc$log2FoldChange,
                            col = list(L1_Gut_LFC = col_fun))
Ha_L1chip_L1gutLFC
```




```
# pdf(file = "./03_plots/200511_AnyDE_Genes_ELT2_ELT7_L1gutLFC.pdf", width = 5, height = 5)
# Ha_L1chip_L1gutLFC
# dev.off()
```

```
RNA_heatmap(dynamic_counts_matrix_scaled_ascend,
            split = elt2_L1_anno$elt2_detected_in_L1) +
  rowAnnotation(L1_Gut_LFC = L1_gut_lfc$log2FoldChange,
                col = list(L1_Gut_LFC = col_fun))
```

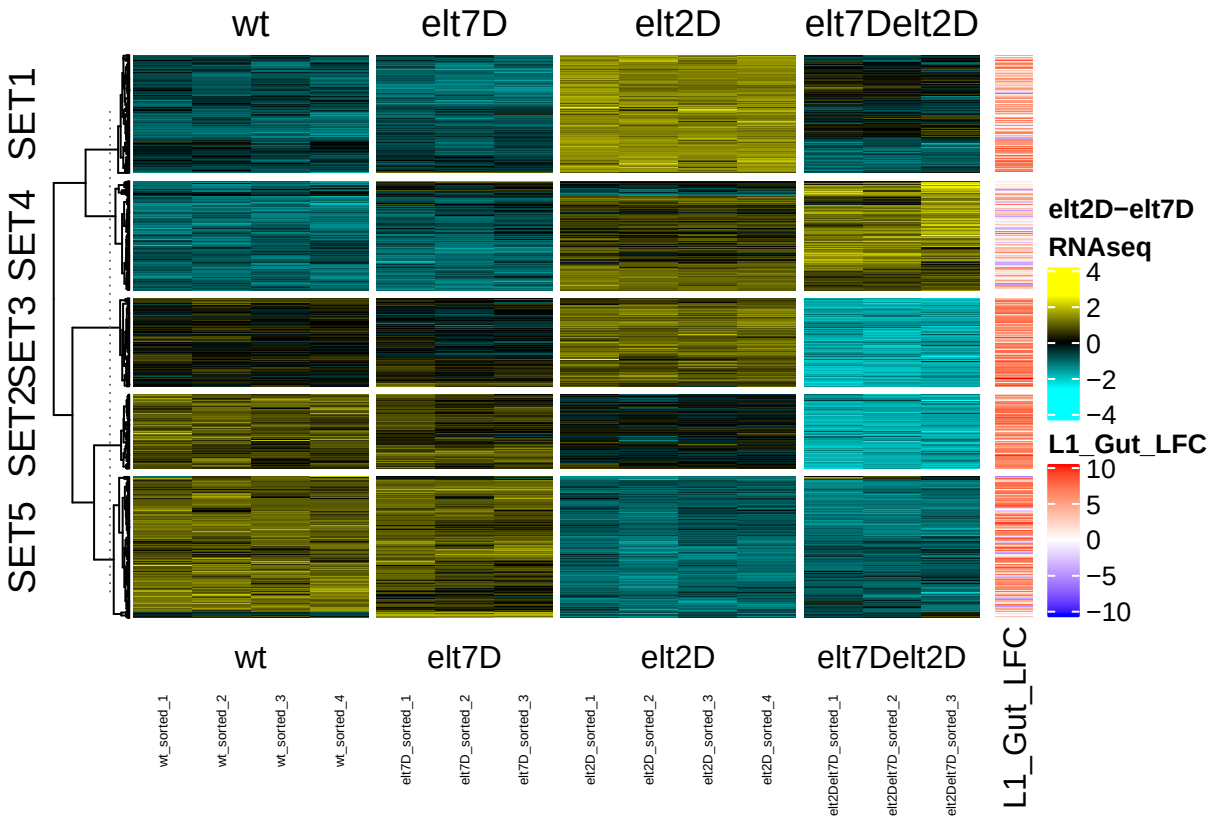


Intestine expression annotation for only ELT-2 bound genes

```
bound_only_L1_gut_lfc <-
  dynamic_counts_matrix_scaled_bound_only %>% as.data.frame.matrix() %>% rownames_to_column(var = "WBGe

identical(
  bound_only_L1_gut_lfc$WBGeneID,
  rownames(dynamic_counts_matrix_scaled_bound_only)
)
```

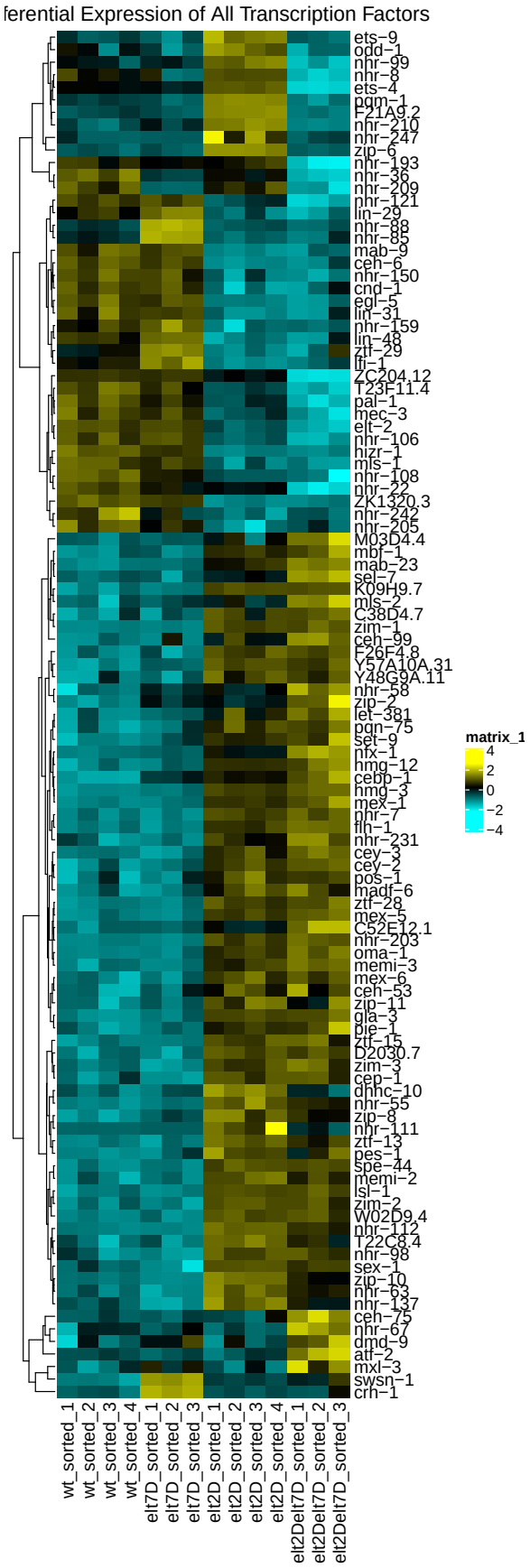
```
## [1] TRUE
```

```
Ha_bound_only + rowAnnotation(L1_Gut_LFC = bound_only_L1_gut_lfc$log2FoldChange,
                              col = list(L1_Gut_LFC = col_fun))
```



Make a TF subset heatmap

```
wTF3.0 <-
  read.csv("./01_input/TF3-0_namesonly.txt",
           sep = "\t",
           header = TRUE) %>% select(WBGeneID)

dynamic_counts_matrix_scaled_TFs <-
  matrix_select(dynamic_counts_matrix_scaled_ascend, wTF3.0$WBGeneID)

dynamic_counts_matrix_scaled_TFs_names <-
  id2name(dynamic_counts_matrix_scaled_TFs)

tf_heatmap <- Heatmap(
  dynamic_counts_matrix_scaled_TFs_names,
  col = colorRampPalette(c("cyan", "black", "yellow"))(1000),
  cluster_columns = FALSE,
  clustering_distance_rows = "spearman",
  clustering_method_rows = "complete",
  show_row_names = TRUE,
  show_column_names = TRUE,
  column_title = "Differential Expression of All Transcription Factors"
)
tf_heatmap
```

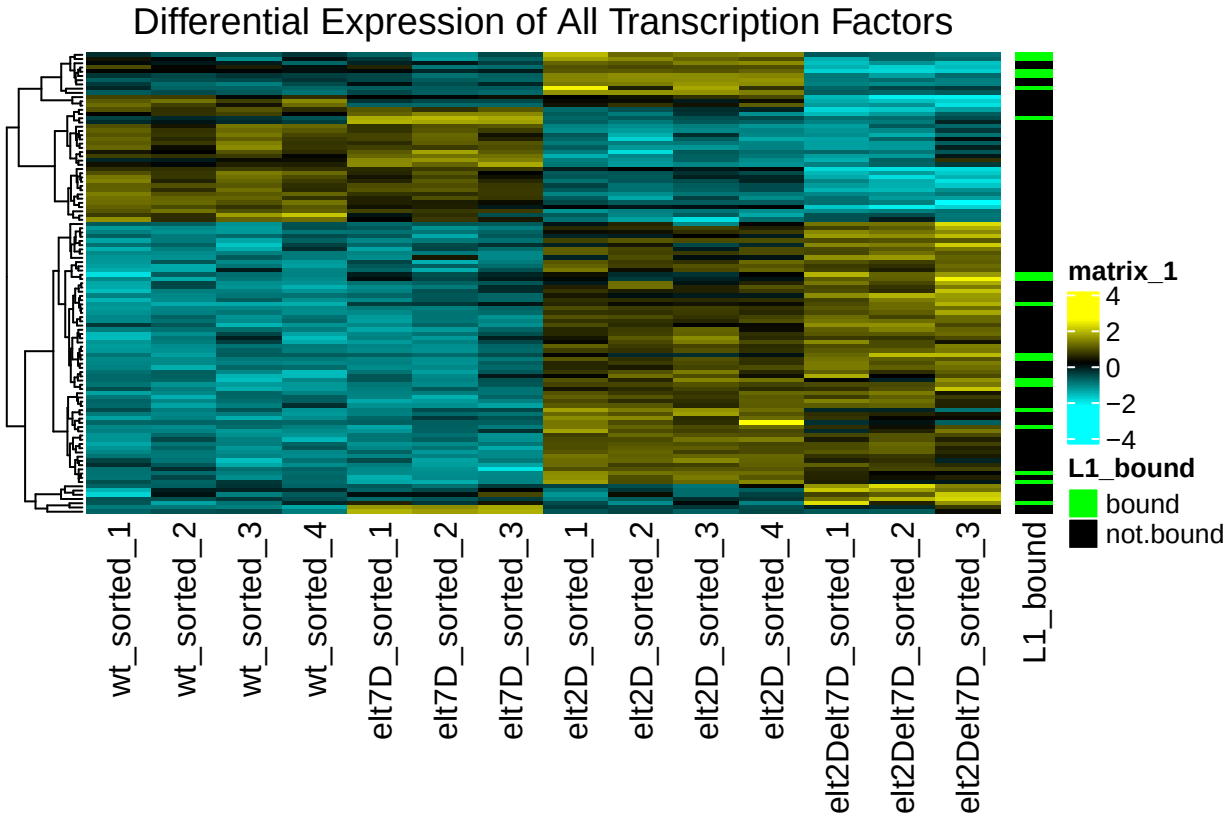

ferential Expression of All Transcription Factors
ets−9
odd−1
nhr−99
nhr−8
ets−4
pqn−1
F21A9.2
nhr−210
nhr−247
zip−6
nhr−193
nhr−36
nhr−209
nhr−121
lin−29
nhr−88
nhr−85
mab−9
ceh−6
nhr−150
cnd−1
egl−5
lin−31
nhr−159
lin−48
ztf−29
ttr−1
ZC204.12
T23F11.4
pal−1
mec−3
elt−2
nhr−106
hizr−1
mls−1
nhr−108
nhr−22
ZK1320.3
nhr−242
nhr−205
M03D4.4
mbf−1
mab−23
sel−7
K09H9.7
mls−2
C38D4.7
zim−1
ceh−99
F26F4.8
Y57A10A.31
Y48G9A.11
nhr−58
zip−2
let−381
pqn−75
set−9
nfx−1
hmg−12
cebp−1
hmg−3
mex−1
pnr−7
flh−1
nhr−231
cey−3
cey−2
pos−1
madf−6
ztf−28
mex−5
C52E12.1
nhr−203
oma−1
memi−3
mex−6
ceh−53
zip−11
gla−3
pie−1
ztf−15
D2030.7
zim−3
cep−1
dhhc−10
nhr−55
zip−8
nhr−111
ztf−13
pes−1
spe−44
memi−2
isl−1
zim−2
W02D9.4
nhr−112
T22C8.4
nhr−98
sex−1
zip−10
nhr−63
nhr−137
ceh−75
nhr−67
dmd−9
atf−2
mxl−3
swsn−1
crh−1
matrix_1
4
2
0
−2
−4
wt_sorted_1
wt_sorted_2
wt_sorted_3
wt_sorted_4
elt7D_sorted_1
elt7D_sorted_2
elt7D_sorted_3
elt2D_sorted_1
elt2D_sorted_2
elt2D_sorted_3
elt2D_sorted_4
elt2Delt7D_sorted_1
elt2Delt7D_sorted_2
elt2Delt7D_sorted_3

Add row annotation to indicate ELT-2 binding in L1 stage

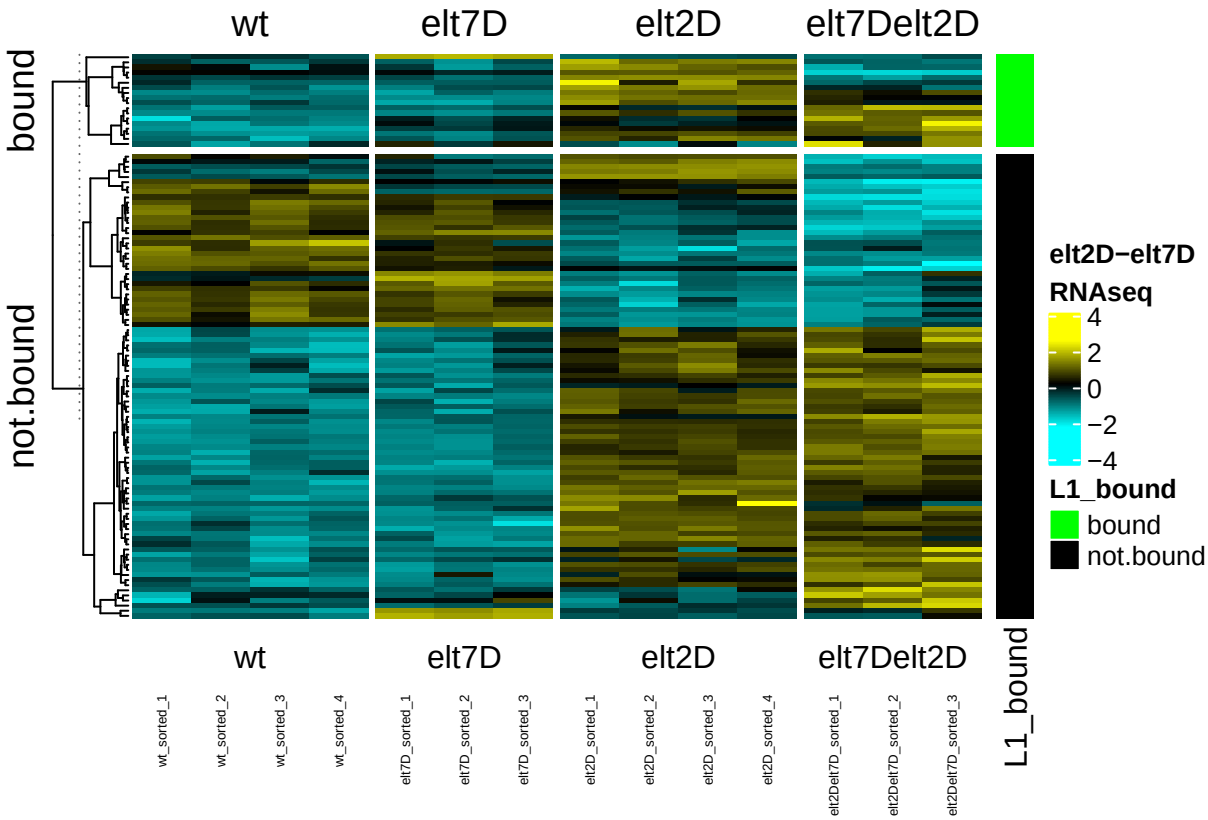
```
elt2_detected_in_L1 %>% filter(WBGeneID %in% rownames(dynamic_counts_matrix_scaled_TFs))
```

```
## # A tibble: 18 x 1
##    WBGeneID
##    <chr>
##  1 WBGene00011376
##  2 WBGene00003678
##  3 WBGene00016888
##  4 WBGene00004096
##  5 WBGene00019327
##  6 WBGene00003845
##  7 WBGene00021082
##  8 WBGene00019743
##  9 WBGene00003648
## 10 WBGene00012101
## 11 WBGene00014193
## 12 WBGene00016997
## 13 WBGene00018704
## 14 WBGene00016865
## 15 WBGene00019344
## 16 WBGene00017687
## 17 WBGene00003727
## 18 WBGene00003511
```

```
tf_bound_anno <-
  data.frame(
    WBGeneID = rownames(dynamic_counts_matrix_scaled_TFs),
    elt2_detected_in_L1 = ifelse(
      test = rownames(dynamic_counts_matrix_scaled_TFs) %in% elt2_detected_in_L1$WBGeneID,
      yes = "bound",
      no = "not.bound"
    )
  )

tf_heatmap_L1bound <-
  tf_heatmap +
  rowAnnotation(L1_bound = tf_bound_anno$elt2_detected_in_L1,
                col = list(L1_bound = c(
                  "bound" = "green", "not.bound" = "black"
                )))
tf_heatmap_L1bound
```

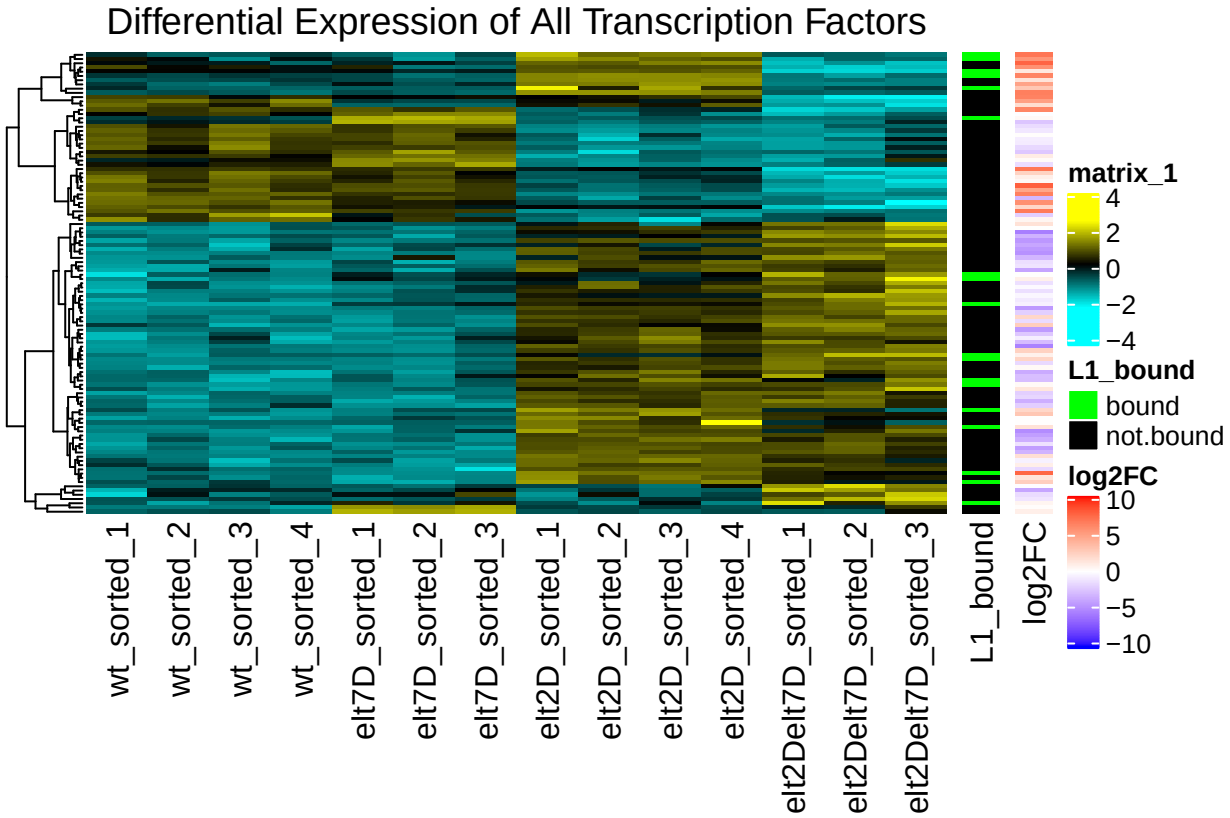



```
RNA_heatmap(dynamic_counts_matrix_scaled_TFs_names,
            split = tf_bound_anno$elt2_detected_in_L1) +
  rowAnnotation(L1_bound = tf_bound_anno$elt2_detected_in_L1,
                col = list(L1_bound = c(
                  "bound" = "green", "not.bound" = "black"
                )))
```

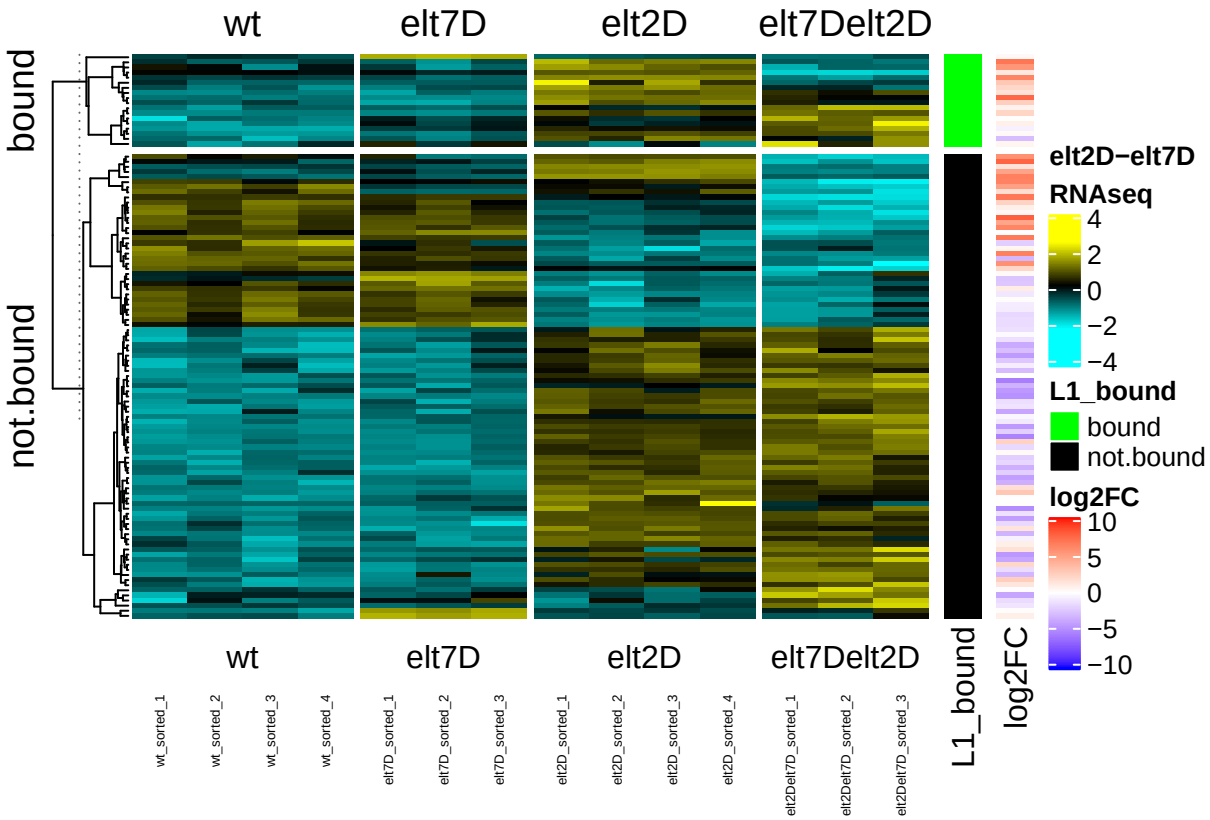


Add row annotation of intestine expression

```
tf_lfc <-
  L1_gut_lfc %>% filter(WBGeneID %in% rownames(dynamic_counts_matrix_scaled_TFs)) %>% select(log2FoldCha

tf_heatmap_L1bound +
  rowAnnotation(log2FC = tf_lfc$log2FoldChange,
                col = list(log2FC = colorRamp2(c(-10, 0, 10), c(
                  "blue", "white", "red"
                ))))
```




```
RNA_heatmap(dynamic_counts_matrix_scaled_TFs_names,
            split = tf_bound_anno$elt2_detected_in_L1) +
  rowAnnotation(L1_bound = tf_bound_anno$elt2_detected_in_L1,
                col = list(L1_bound = c(
                  "bound" = "green", "not.bound" = "black"
                ))) +  rowAnnotation(log2FC = tf_lfc$log2FoldChange,
                                     col = list(log2FC = colorRamp2(c(-10, 0, 10), c(
                                       "blue", "white", "red"
                                     ))))
```



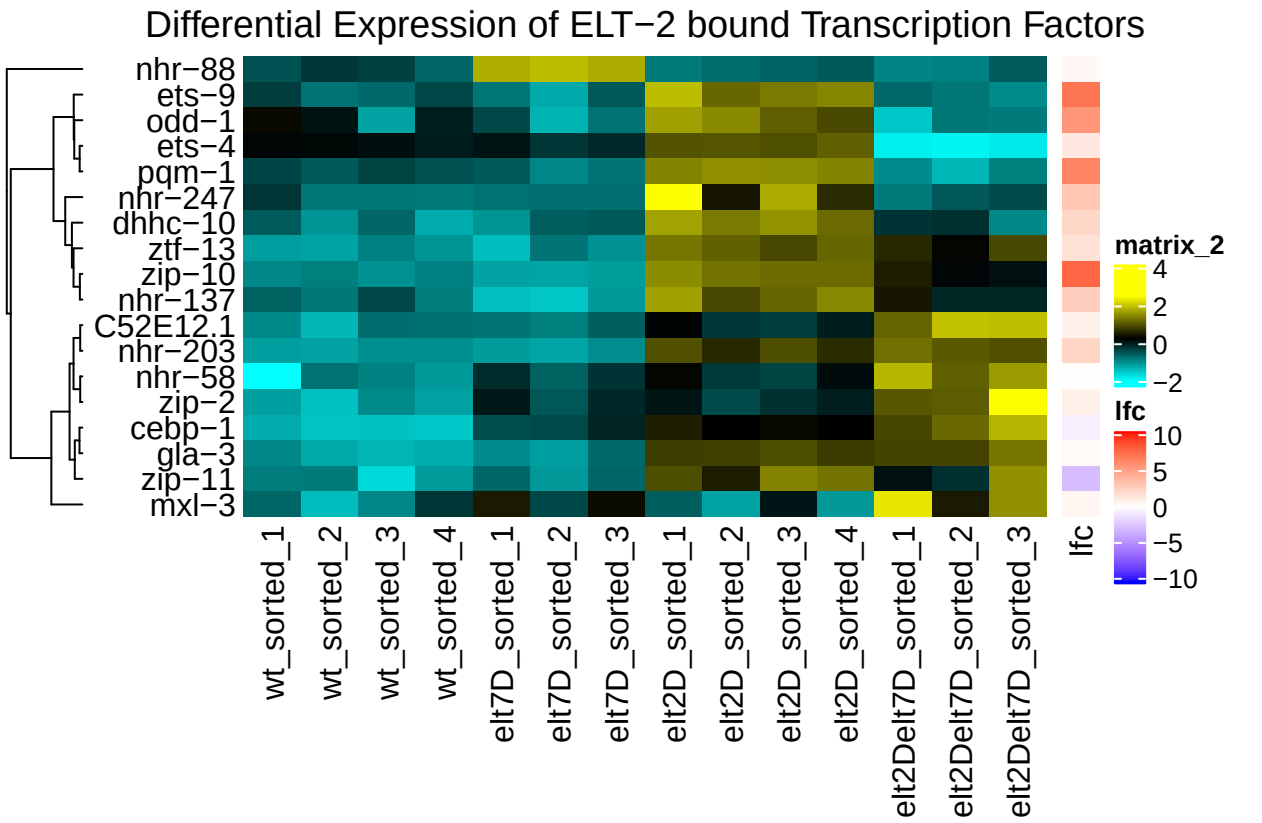
Zoom in on only bound TFs

```
dynamic_counts_matrix_scaled_TFs_bound <-
  matrix_select(dynamic_counts_matrix_scaled_TFs,
                elt2_detected_in_L1$WBGeneID)

dynamic_counts_matrix_scaled_TFs_bound_names <-
  id2name(dynamic_counts_matrix_scaled_TFs_bound)

bound_tf_lfc <-
  L1_gut_lfc %>% filter(WBGeneID %in% rownames(dynamic_counts_matrix_scaled_TFs_bound)) %>% select(log2

head(bound_tf_lfc)
```

```
##   log2FoldChange
## 1     0.53748793
## 2     0.07431616
## 3     0.34541162
## 4     2.53692129
## 5     5.37850676
## 6     6.24020573
```

```
Heatmap(
  dynamic_counts_matrix_scaled_TFs_bound_names,
  col = colorRampPalette(c("cyan", "black", "yellow"))(1000),
  cluster_columns = FALSE,
  clustering_distance_rows = "spearman",
  clustering_method_rows = "complete",
  show_row_names = TRUE,
```

```
  row_names_side = "left",
  show_column_names = TRUE,
  column_title = "Differential Expression of ELT-2 bound Transcription Factors"
) +
  rowAnnotation(lfc = bound_tf_lfc$log2FoldChange,
                col = list(lfc = colorRamp2(c(-10, 0, 10), c(
                  "blue", "white", "red"
                ))))
```



This plot suggests that transcription factors bound by ELT-2 are typically upregulated in the absence of ELT-2.

Additionally, TFs that are expressed in the L1 intestine are upregulated in absence of ELT-2 alone, but downregulated in the absence of both ELT-2 and ELT-7.

Futhermore, TFs that are not expressed in the L1 intestine are upregulated only in the absence of both ELT-2 and ELT-7.

TFs to follow up: pqm-1, zip-10, odd-1 (repressed by elt-2 alone, normally gut expressed). nhr-58 (vulva), zip-2 (neuron), cebp-1 (neuron), gla-3 (germline), zip-11

old code below

Transcription factor subset plots

Results and interpretation

Session Info

```
sessionInfo()
```

```
## R version 3.6.3 (2020-02-29)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS High Sierra 10.13.6
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/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_0.4.9       binom_1.1-1          dendextend_1.13.4
##  [4] RVAideMemoire_0.9-75 pheatmap_1.0.12      matrixStats_0.56.0
##  [7] ComplexHeatmap_2.2.0 readxl_1.3.1         forcats_0.5.0
## [10] stringr_1.4.0        dplyr_0.8.5          purrr_0.3.4
## [13] readr_1.3.1          tidyr_1.0.3          tibble_3.0.1
## [16] ggplot2_3.3.0        tidyverse_1.3.0      biomaRt_2.42.1
##
## loaded via a namespace (and not attached):
##  [1] nlme_3.1-147         fs_1.4.1             lubridate_1.7.8
##  [4] bit64_0.9-7          RColorBrewer_1.1-2   progress_1.2.2
##  [7] httr_1.4.1           tools_3.6.3          backports_1.1.6
## [10] utf8_1.1.4           R6_2.4.1             DBI_1.1.0
## [13] BiocGenerics_0.32.0  colorspace_1.4-1     GetoptLong_0.1.8
## [16] withr_2.2.0          gridExtra_2.3        tidyselect_1.0.0
## [19] prettyunits_1.1.1    bit_1.1-15.2         curl_4.3
## [22] compiler_3.6.3       cli_2.0.2            rvest_0.3.5
## [25] Biobase_2.46.0       xml2_1.3.2           labeling_0.3
## [28] scales_1.1.0         askpass_1.1          rappdirs_0.3.1
## [31] digest_0.6.25        rmarkdown_2.1        pkgconfig_2.0.3
## [34] htmltools_0.4.0      dbplyr_1.4.3         rlang_0.4.6
## [37] GlobalOptions_0.1.1  rstudioapi_0.11      RSQLite_2.2.0
## [40] farver_2.0.3         shape_1.4.4          generics_0.0.2
## [43] jsonlite_1.6.1       magrittr_1.5         Rcpp_1.0.4.6
## [46] munsell_0.5.0        S4Vectors_0.24.4     fansi_0.4.1
## [49] viridis_0.5.1        lifecycle_0.2.0      stringi_1.4.6
## [52] yaml_2.2.1           BiocFileCache_1.10.2 blob_1.2.1
## [55] parallel_3.6.3       crayon_1.3.4         lattice_0.20-41
## [58] haven_2.2.0          hms_0.5.3            knitr_1.28
## [61] pillar_1.4.4         rjson_0.2.20         stats4_3.6.3
```

```
## [64] reprex_0.3.0          XML_3.99-0.3          glue_1.4.0
## [67] evaluate_0.14         modelr_0.1.7          png_0.1-7
## [70] vctrs_0.2.4           cellranger_1.1.0      gtable_0.3.0
## [73] openssl_1.4.1         clue_0.3-57           assertthat_0.2.1
## [76] xfun_0.13             broom_0.5.6           viridisLite_0.3.0
## [79] AnnotationDbi_1.48.0 memoise_1.1.0         IRanges_2.20.2
## [82] cluster_2.1.0         ellipsis_0.3.0
```