RWC23_ELT2_Regulated_Genes

RTPW

4/13/2020

Decide if plots should be saved to files:

Change `plot` to `TRUE` if you want to write plots to a file change `plot` to `FALSE` if you do not want to write plots to a file

```
plot <- FALSE
plotdir <- "./03_plots/"
```

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)
```

```
## -- Attaching packages ---------------------------------------------------------------------------
## v ggplot2 3.3.0     v purrr   0.3.4
## v tibble  3.0.1     v dplyr   0.8.5
## v tidyr   1.0.3     v stringr 1.4.0
## v readr   1.3.1     v forcats 0.5.0
## -- Conflicts ------------------------------------------------------------------------------------
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
## x dplyr::select() masks biomaRt::select()
```

```
library(readxl)
library(ComplexHeatmap)
```

```
## Loading required package: grid
```

```
## ========================================
## ComplexHeatmap version 2.2.0
## 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.
## ========================================
```

```
library(matrixStats)
```

```
##
## Attaching package: 'matrixStats'
```

```
## The following object is masked from 'package:dplyr':
##
##     count
```

```
library(pheatmap)
library(RVAideMemoire)
```

```
## *** Package RVAideMemoire v 0.9-75 ***
```

```
library(dendextend)
```

```
##
## ---------------------
## Welcome to dendextend version 1.13.4
## Type citation('dendextend') for how to cite the package.
##
## Type browseVignettes(package = 'dendextend') for the package vignette.
## The github page is: https://github.com/talgalili/dendextend/
##
## Suggestions and bug-reports can be submitted at: https://github.com/talgalili/dendextend/issues
## Or contact: <tal.galili@gmail.com>
##
##  To suppress this message use:  suppressPackageStartupMessages(library(dendextend))
## ---------------------
```

```
##
## Attaching package: 'dendextend'
```

```
## The following object is masked from 'package:stats':
##
##     cutree
```

```
library(binom)
library(circlize)
```

```
## ========================================
## circlize version 0.4.9
## 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))
## ========================================
```

```
library(lubridate)
```

```
##
## Attaching package: 'lubridate'
```

```
## The following objects are masked from 'package:dplyr':
##
##     intersect, setdiff, union
```

```
## The following objects are masked from 'package:base':
##
##     date, intersect, setdiff, union
```

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

Description of Data

I will integrate a RNA-seq experiment, a microarray experiment 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 dataset is from a 2011 paper using FACS sorting of Late Embryo (LE) and Larval Stage 2 (L2) intestine cells, measured with microarray. See Spencer et. al, (2011).

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

3) Spencer, W. C., Zeller, G., Watson, J. D., Henz, S. R., Watkins, K. L., McWhirter, R. D., Petersen, S., Sreedharan, V. T., Widmer, C., Jo, J., Reinke, V., Petrella, L., Strome, S., Von Stetina, S. E., Katz, M., Shaham, S., Rätsch, G., & Miller, D. M. (2011). A spatial and temporal map of C. elegans gene expression. Genome Research, 21(2), 325–341. https://doi.org/10.1101/gr.114595.110

4) Boeck, M. E., Huynh, C., Gevirtzman, L., Thompson, O. A., Wang, G., Kasper, D. M., Reinke, V., Hillier, L. W., & Waterston, R. H. (2016). The time-resolved transcriptome of C. elegans. Genome Research, 26(10), 1441–1450. https://doi.org/10.1101/gr.202663.115

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 = "./01_input/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
```

Load differential expression clusters from Dineen and Nishimura et al (2018).

```
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_GRange <- readRDS("./01_input/200719_annotatedPeaks.rds")
# elt2_peaks <- mcols(elt2_GRange) %>% as.data.frame() %>% remove_rownames()
# elt2_peaks
# elt2_peaks <- elt2_peaks %>% rename(cluster.description = k4labels, WBGeneID = feature)
# write_csv(elt2_peaks,"./01_input/200719_annotatedPeaks.csv")
```

```
elt2_peaks <- read_csv(file = "./01_input/200719_annotatedPeaks.csv")
```

```
## Parsed with column specification:
## cols(
##   .default = col_double(),
##   name = col_character(),
##   cluster.description = col_character(),
##   peak = col_character(),
##   WBGeneID = col_character(),
##   feature_strand = col_character(),
##   insideFeature = col_character(),
##   fromOverlappingOrNearest = col_character()
## )
```

```
## See spec(...) for full column specifications.
```

```
elt2_peaks$cluster.description <-
  factor(
    elt2_peaks$cluster.description,
```

```
    levels = 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 32
##    LE_1  LE_2  L1_1  L1_2  L3_1  L3_2 LE_IDR L1_IDR L3_IDR summit_agreement
##   <dbl> <dbl> <dbl> <dbl> <dbl> <dbl>  <dbl>  <dbl>  <dbl>            <dbl>
## 1  1.93  1.60  4.25  3.77  4.88  5.01      0      1      1             27.4
## 2  2.11  1.94  4.05  4.46  4.95  5.94      0      1      1             12.4
## 3  1.22  1.53  2.61  2.85  2.45  2.86      0      0      1            137
## 4  1.81  1.42  2.74  3.28  4.18  4.49      0      0      1              2.5
## 5  2.22  2.17  2.24  2.13  4.02  4.10      1      1      1             10
## 6  1.89  2.10  3.43  2.85  3.42  3.53      0      0      1            124.
## # ... with 22 more variables: k4cluster <dbl>, k11cluster <dbl>,
## #   k4weights <dbl>, k11weights <dbl>, LE_nonNormed <dbl>, L1_nonNormed <dbl>,
## #   L3_nonNormed <dbl>, LE_std <dbl>, L1_std <dbl>, L3_std <dbl>, name <chr>,
## #   cluster.description <fct>, variance <dbl>, peak <chr>, WBGeneID <chr>,
## #   start_position <dbl>, end_position <dbl>, feature_strand <chr>,
## #   insideFeature <chr>, distancetoFeature <dbl>, shortestDistance <dbl>,
## #   fromOverlappingOrNearest <chr>
```

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

```
elt2_detected_in_L1 <-
  elt2_peaks %>% select(WBGeneID, L1_IDR) %>% filter(L1_IDR == 1) %>% select(WBGeneID) %>% unique()

elt2_detected_in_L1 %>% head
```

```
## # A tibble: 6 x 1
##   WBGeneID
##   <chr>
## 1 WBGene00022277
## 2 WBGene00022276
## 3 WBGene00021026
## 4 WBGene00022037
## 5 WBGene00022042
## 6 WBGene00007009
```

```
elt2_detected_in_L1 %>% dim
```

```
## [1] 2416    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
## WBGene00000004               0      2          0       0            0
## WBGene00000007               0      0          2       0            0
## WBGene00000008               0      0          1       0            0
## WBGene00000009               0      1          0       0            0
## WBGene00000018               0      0          0       1            0
## WBGene00000022               0      0          0       1            0
```

Load Spencer et. al. intestine expression

This data is from a 2011 paper using FACS sorting of Late Embryo (LE) and Larval Stage 2 (L2) intestine cells, measured with microarray. See Spencer et. al, (2011).

```
spencerLEgenes <-
  read.table(
    "./01_input/Spencer_et_al_2010_FACS_and_pulldown_tilling_array/LE-intestine_enr_vs_ref.WS200.txt",
    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(
    "./01_input/Spencer_et_al_2010_FACS_and_pulldown_tilling_array/L2-intestine_enr_vs_ref.WS200.txt",
    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
```

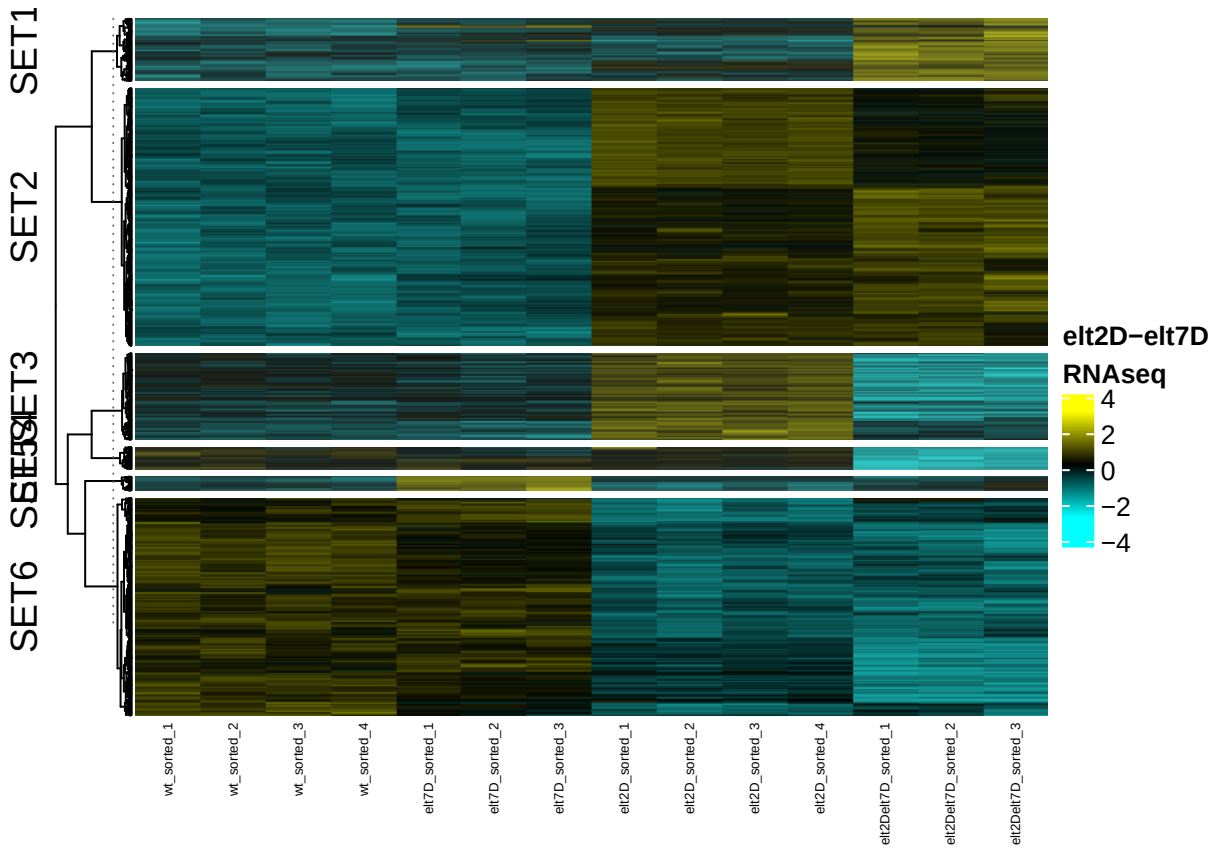
```
dynamic_counts_matrix_scaled_ascend <-
  dynamic_counts_matrix_scaled[order(rownames(dynamic_counts_matrix_scaled)),]
```

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
)
```



Add expression set and column labels.

```
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
```

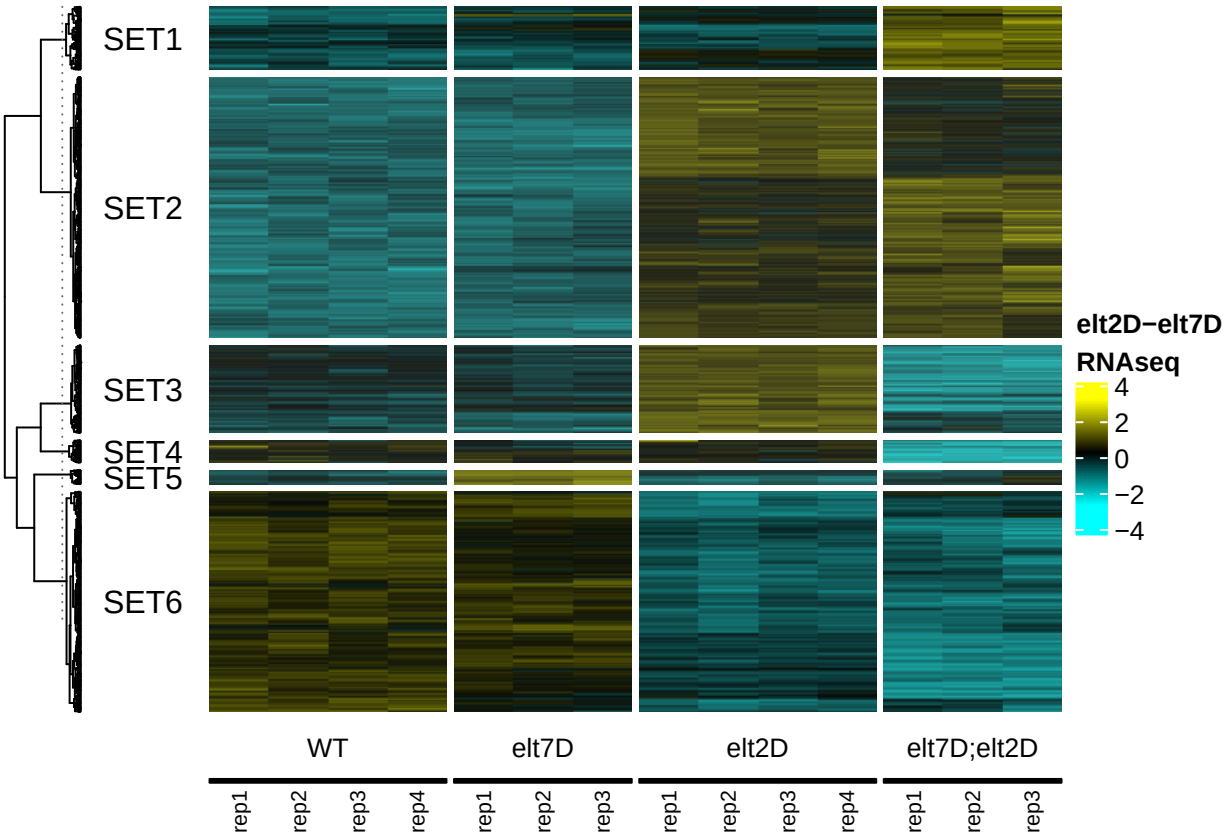
```
##  [1] WT          WT          WT          WT          elt7D       elt7D
##  [7] elt7D       elt2D       elt2D       elt2D       elt2D       elt7Delt2D
## [13] elt7Delt2D elt7Delt2D
## Levels: WT elt7D elt2D elt7Delt2D
```

```
column_labels <-
  structure(
    c(
      "rep1",
      "rep2",
      "rep3",
      "rep4",
      "rep1",
      "rep2",
      "rep3",
      "rep1",
```

```
      "rep2",
      "rep3",
      "rep4",
      "rep1",
      "rep2",
      "rep3"
    ),
    names = colnames(dynamic_counts_matrix_scaled_ascend)
  )

column_labels
```

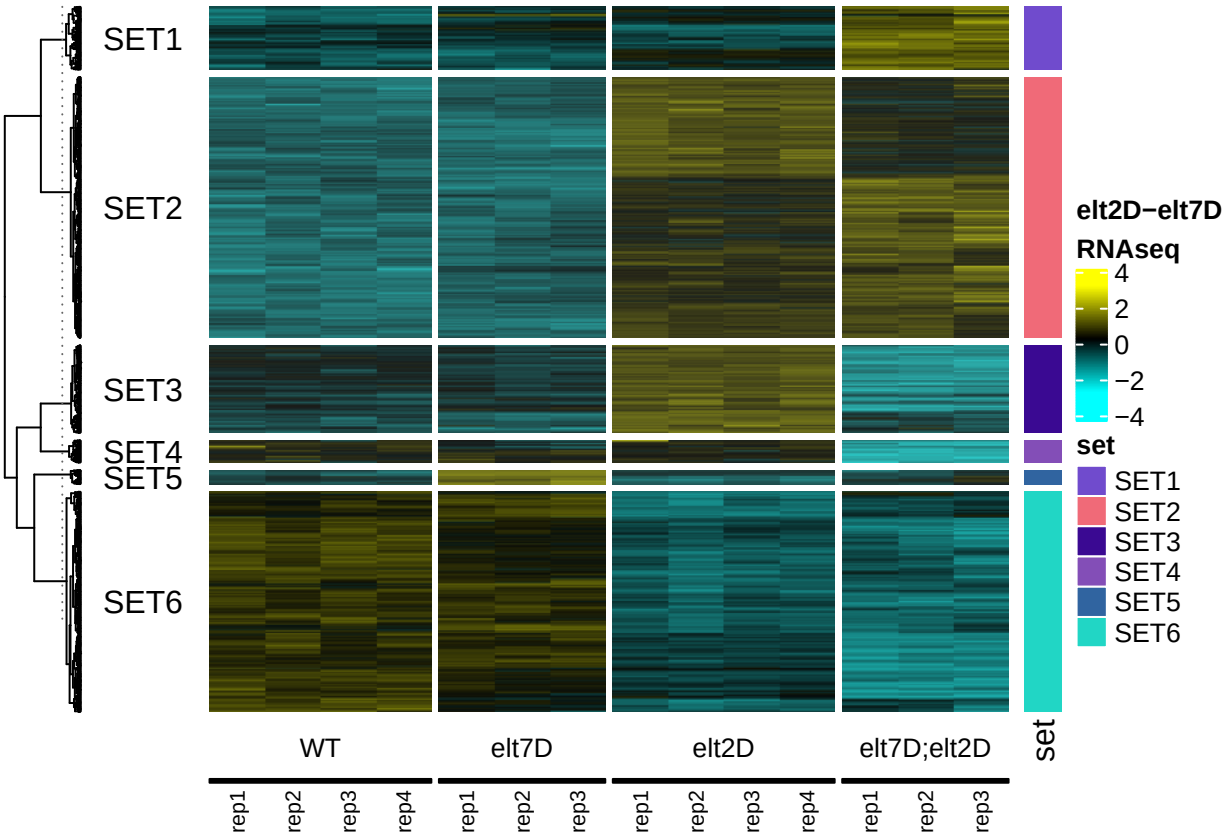
```
##         wt_sorted_1         wt_sorted_2         wt_sorted_3         wt_sorted_4
##              "rep1"              "rep2"              "rep3"              "rep4"
##      elt7D_sorted_1      elt7D_sorted_2      elt7D_sorted_3      elt2D_sorted_1
##              "rep1"              "rep2"              "rep3"              "rep1"
##      elt2D_sorted_2      elt2D_sorted_3      elt2D_sorted_4 elt2Delt7D_sorted_1
##              "rep2"              "rep3"              "rep4"              "rep1"
## elt2Delt7D_sorted_2 elt2Delt7D_sorted_3
##              "rep2"              "rep3"
```

```
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,
  column_labels = column_labels[colnames(dynamic_counts_matrix_scaled_ascend)],
  column_names_gp = gpar(cex = 0.7),
  heatmap_legend_param = list(color_bar = "continuous"),
  row_split = dineen_nishimura_sets_ascend$set,
  row_title = NULL,
  column_title = NULL,
  column_split = RNA_column_order,
  bottom_annotation = HeatmapAnnotation(
    foo = anno_block(
      labels = c("WT", "elt7D", "elt2D", "elt7D;elt2D"),
      labels_gp = gpar(cex = .8),
      gp = gpar(border = NA, lty = "blank")
      ),
    foo2 = anno_block(gp = gpar(fill = "black"), height = unit(0.5, "mm"))
  ),
  left_annotation = rowAnnotation(foo = anno_block(
    labels = c("SET1", "SET2", "SET3", "SET4", "SET5", "SET6"),
    labels_rot = 0,
    gp = gpar(border = NA, lty = "blank", cex = 0.4)
  ))
)
Ha
```



Sanity check to ensure that cluster splitting is occuring correctly. Remap the Set assignments back to the heatmap as a row annotation.

```
Ha + rowAnnotation(set = dineen_nishimura_sets_ascend$set)
```



Add L1 stage ELT-2 binding

This section will add annotation to the rows of the elt2/elt7 differentiall expression heatmap with ELT-2 ChIP-seq binding during the L1 stage. This will determine what differential expression sets associate with ELT-2 binding during the L1 stage. The reason L1 stage ChIP-seq eaks are being used is because the elt2/elt7 RNA-seq experiment was conducted in the L1 stage.

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

```
elt2_detected_in_L1 %>% dim
```

```
## [1] 2416    1
```

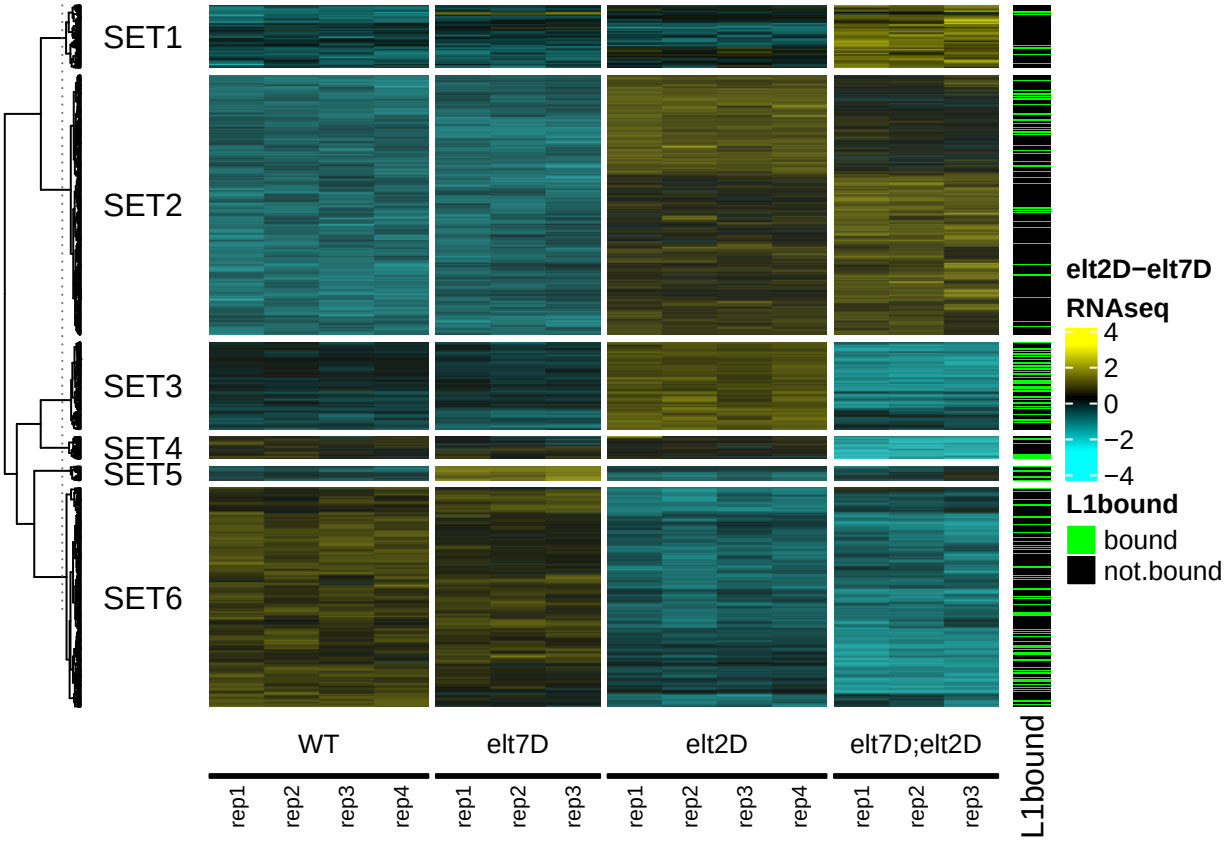
```
elt2_L1_anno <-
  data.frame(
    WBGeneID = rownames(dynamic_counts_matrix_scaled_ascend),
    elt2_detected_in_L1 = ifelse(
      test = rownames(dynamic_counts_matrix_scaled_ascend) %in% elt2_detected_in_L1$WBGeneID,
      yes = "bound",
      no = "not.bound"
    ),
    stringsAsFactors = FALSE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

elt2_L1_anno %>% head()
```

```
##         WBGeneID elt2_detected_in_L1
## 1 WBGene00000007               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
```

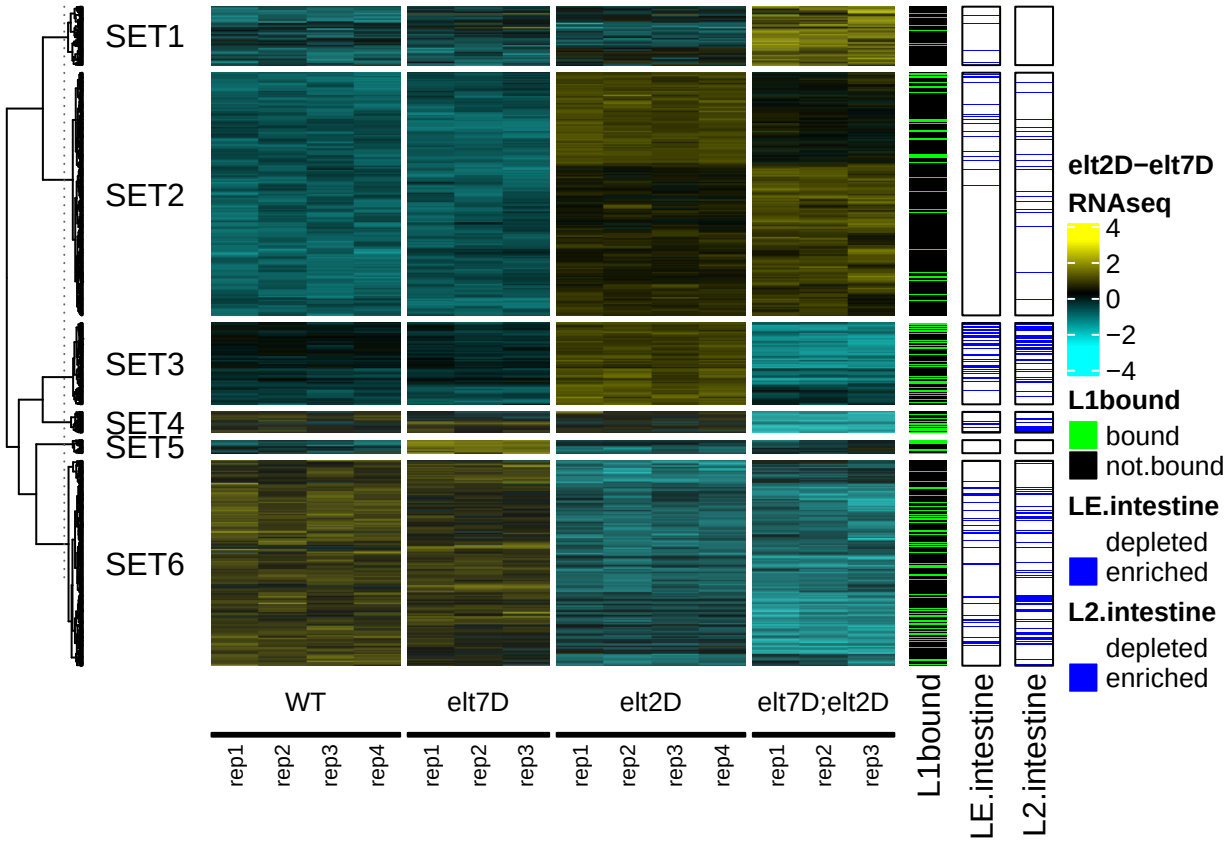



```
if (plot == TRUE){
myPDFplot(Ha_L1chip, "01a_DE_Heatmap_elt2elt7DERNAseq_L1elt2bound", 4, 4.5, plotdir)
}
```

Add Spencer intestine data

```
spencer_rna_anno <- data.frame(
  spencerLE = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_ascend) %in% spencer_LE_subset$spencer_LE_ID,
    yes = "enriched",
    no = "depleted"
  ),
  spencerL2 = ifelse(
```

```
    test = rownames(dynamic_counts_matrix_scaled_ascend) %in% spencer_L2_subset$spencer_L2_ID,
    yes = "enriched",
    no = "depleted"
  )
)

Ha_L1chip_spencer <- Ha_L1chip +
  rowAnnotation(
    LE.intestine = spencer_rna_anno$spencerLE,
    col = list(LE.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  ) +
  rowAnnotation(
    L2.intestine = spencer_rna_anno$spencerL2,
    col = list(L2.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  )

Ha_L1chip_spencer
```




```
if (plot == TRUE) {
  myPDFplot(Ha_L1chip_spencer, "01b_DE_Heatmap_elt2elt7DERNAseq_L1elt2bound_spencerRNA", height = 6.5, 
}
```

Visually it appears that some elt2/elt7 differential expression clusters have more or less ELT-2 binding associated with the sets. I would like to be more quantitative with this assesment.

Determine enrichment of ELT-2 binding during L1 stage. I will calculate the percentage of genes with an ELT-2 ChIP-seq peak detected during the L1 stage.

First use `merge` to combine the ELT-2 binding status and expression set for each gene.

```
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               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
```

Next use `table` to tally the number of bound and not.bound genes per expression set.

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

```
##
##        bound not.bound
##   SET1    31       260
##   SET2   163      1045
##   SET3   173       232
##   SET4    37        66
##   SET5    17        48
##   SET6   251       769
```

Use `prop.table` to convert these values to percentages within each set.

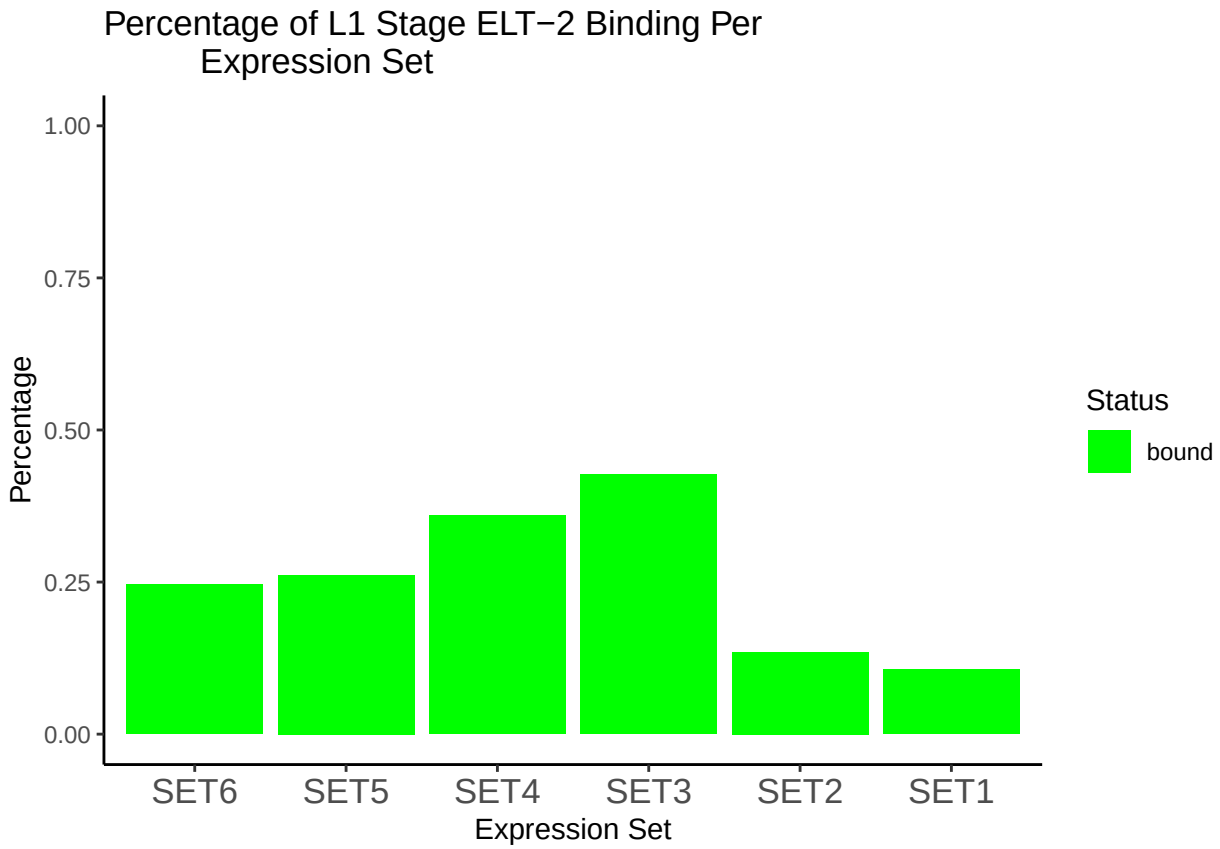
```
clust_L1bound_prop <- prop.table(clust_L1bound_counts, 1)
clust_L1bound_prop
```

```
##
##            bound not.bound
##   SET1 0.1065292 0.8934708
##   SET2 0.1349338 0.8650662
##   SET3 0.4271605 0.5728395
##   SET4 0.3592233 0.6407767
##   SET5 0.2615385 0.7384615
##   SET6 0.2460784 0.7539216
```

Adjust the percentages object into a dataframe that `ggplot2` can use.

```
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
```



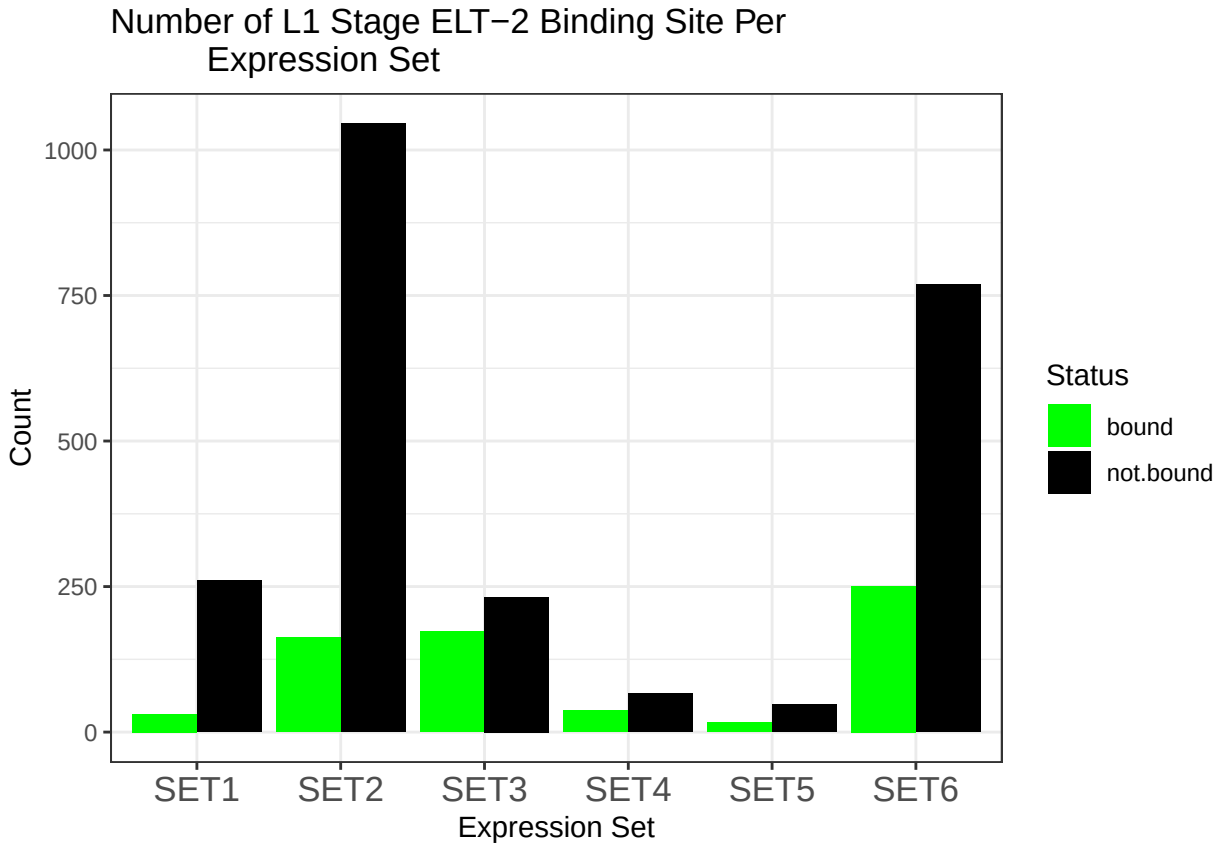

```
if (plot == TRUE){
myggsave(plot = l1bound_percents,
         name = "02_proportion_of_l1elt2_per_expression_cluster_200428",
         height = 2,
         width = 5,
         plotdir = plotdir)
}
```

This plot shows that all of the differential expression sets have less than 50% of genes bound by ELT-2.

Rather than viewing percentages of genes bound, what is 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")

bound_per_cluster <- 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))
```

```
bound_per_cluster
```




```
if (plot == TRUE){
myggsave(
  plot = bound_per_cluster,
  name = "03_number_of_l1elt2_per_expression_cluster",
  height = 2,
  width = 5,
  plotdir = plotdir
  )
}
```

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] 0.2173351
```

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 1.089477e-06 0.07353755  0.1477938  TRUE
## 2 SET2 3.090100e-13 0.11616023  0.1555038  TRUE
## 3 SET3 3.491899e-21 0.37843660  0.4769565  TRUE
## 4 SET4 1.095787e-03 0.26704257  0.4597282  TRUE
## 5 SET5 3.695287e-01 0.16032876  0.3853937 FALSE
## 6 SET6 2.765586e-02 0.21991907  0.2737118  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.

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

```
##    Set         pval conf.lower conf.upper  bool
## 1 SET1 5.771887e-07          0  0.1410586  TRUE
## 2 SET2 1.554820e-13          0  0.1521773  TRUE
## 3 SET3 1.000000e+00          0  0.4691260 FALSE
## 4 SET4 9.996741e-01          0  0.4441844 FALSE
## 5 SET5 8.449320e-01          0  0.3660888 FALSE
## 6 SET6 9.873319e-01          0  0.2692809 FALSE
```

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

Now try `greater`.

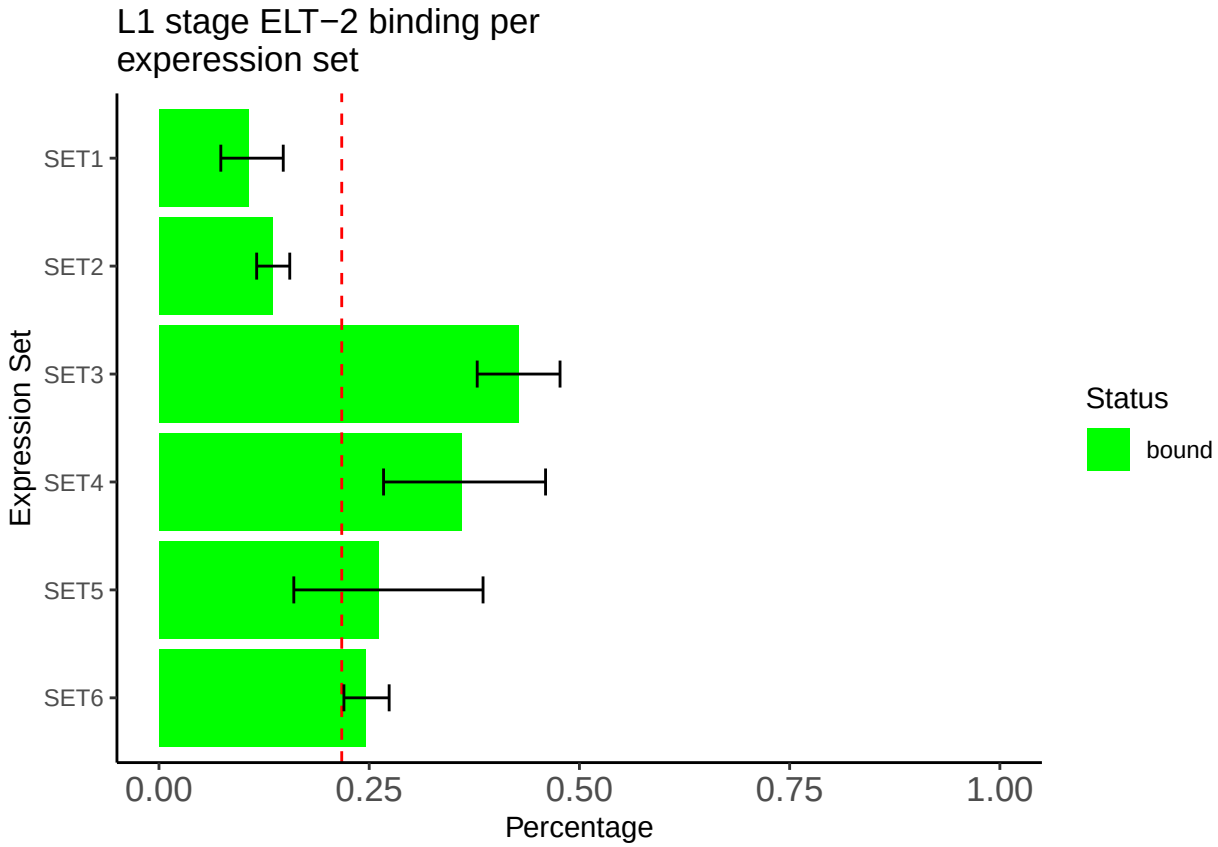
```
ctable_binom(clust_L1bound_counts, "greater")
```

```
##    Set         pval conf.lower conf.upper  bool
## 1 SET1 9.999998e-01 0.07816909          1 FALSE
## 2 SET2 1.000000e+00 0.11902217          1 FALSE
## 3 SET3 3.013813e-21 0.38599089          1  TRUE
## 4 SET4 6.982464e-04 0.28048601          1  TRUE
## 5 SET5 2.334473e-01 0.17401164          1 FALSE
## 6 SET6 1.528872e-02 0.22396830          1  TRUE
```

This says that SET3, SET4 and SET6 have a higher percentage of genes bound compared the the "background" percent of bound genes for the entire dataset.

Make a plot that visually depicts this. Draw line on the percentage plot to indicate background percentage of L1 stage ELT-2 binding.

```
l1bound_percents_verticle <- 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() +
  ggtitle("L1 stage ELT-2 binding per\nexperession set")

l1bound_percents_verticle
```




```
if (plot == TRUE){
myggsave(
  plot = l1bound_percents_verticle,
  name = "04_percentage_l1bound_per_expression_cluster",
  width = 4,
  height = 5,
  plotdir = plotdir
)
}
```

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] 3.957929e-93
```

A very small p-value for the hypergeometric test suggests that the entire dataset is enriched for ELT-2.

The next section with compute pairwise fisher's exact tests for the different sets. I have a difficult time interpreting these results.

```
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 3.332e-20 1.647e-31         -      -    -
## SET4 5.641e-07 1.004e-06 1.000e+00      -    -
## SET5 3.200e-02 1.370e-01 2.077e-01 1.0000    -
## SET6 1.161e-06 3.066e-10 6.302e-10 0.2603    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  TRUE FALSE FALSE FALSE    NA
## SET6  TRUE  TRUE  TRUE FALSE FALSE
```

Row annotation of ELT-2 Binding Pattern Clusters

This section will add annotation to the rows of the elt2/elt7 differentiall expression heatmap with ELT-2 ChIP-seq binding pattern clusters. This will determine what differential expression sets associate with ELT-2 binding patters.

Start by using custom function `make_cluster_annotation()`. This function takes two objects: the matrix of gene expression values and a dataframe of counts ELT-2 binding patterns per genes. It returns a dataframe with the number of ELT-2 binding categories associated with each gene.

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

chip_annotation %>% head()
```

```
##         WBGeneID Embryo_Specific Larval Increasing L3_High Not_Changing
## 1 WBGene00000007               0      0          2       0            0
## 2 WBGene00000008               0      0          1       0            0
## 3 WBGene00000009               0      1          0       0            0
## 4 WBGene00000013               0      0          0       0            0
## 5 WBGene00000016               0      0          0       0            0
## 6 WBGene00000017               0      0          0       0            0
```

Sanity check to ensure that the order and number of rows is preserved.

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

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

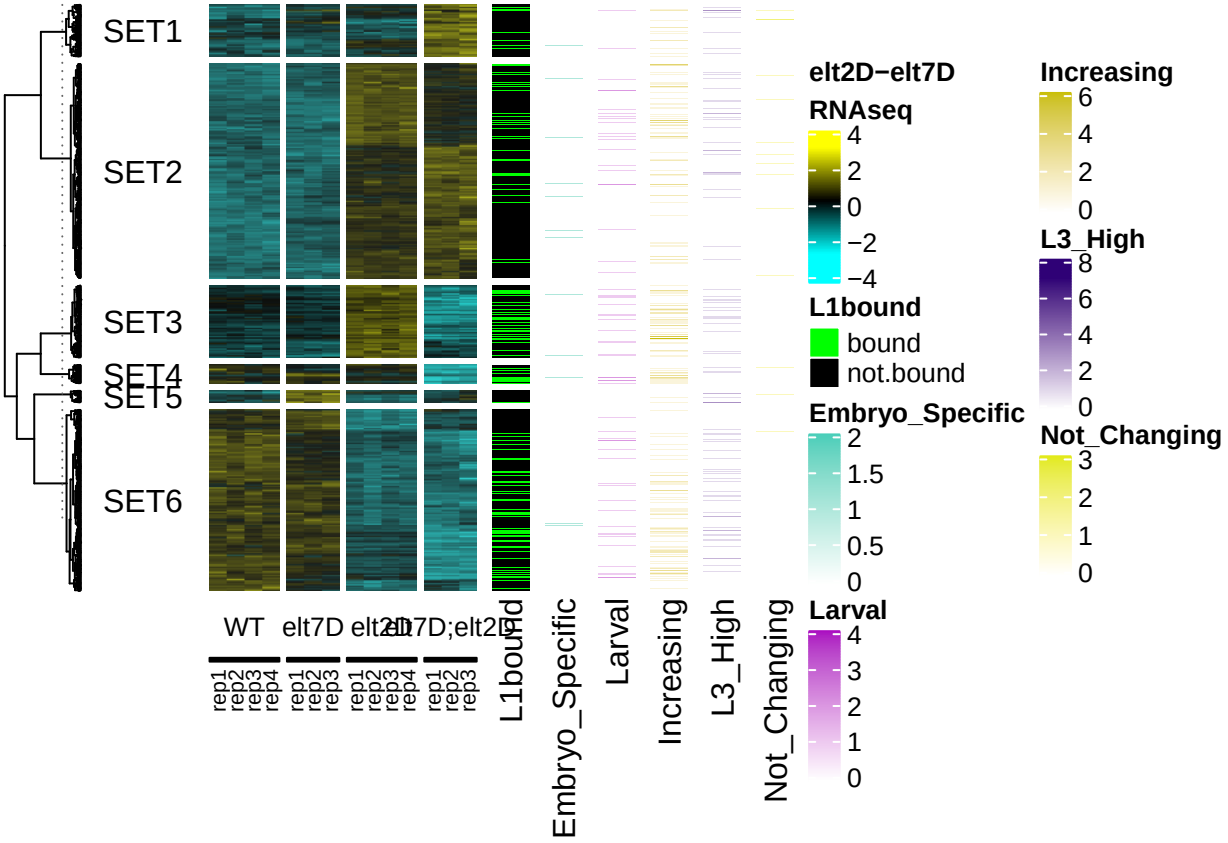
```
nrow(dynamic_counts_matrix_scaled) == nrow(chip_annotation)
```

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

Build add row annotation for the number of ELT-2 binding clusters associated with each gene.

```
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.

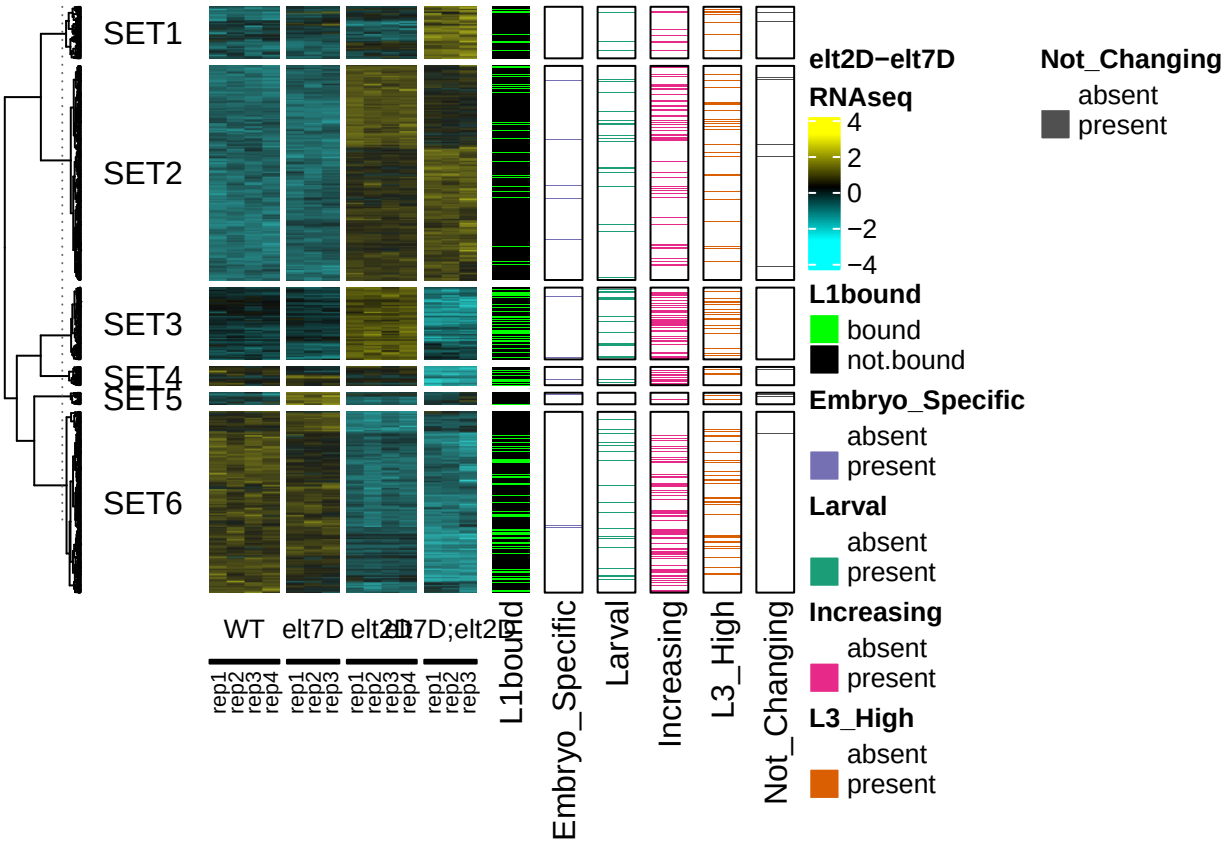
```
cluster_colors <-
  data.frame(
    class = elt2_cluster_names,
    val = c("#7570B3", "#1B9E77", "#E7298A", "#D95F02", "#505050")
  )

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
```

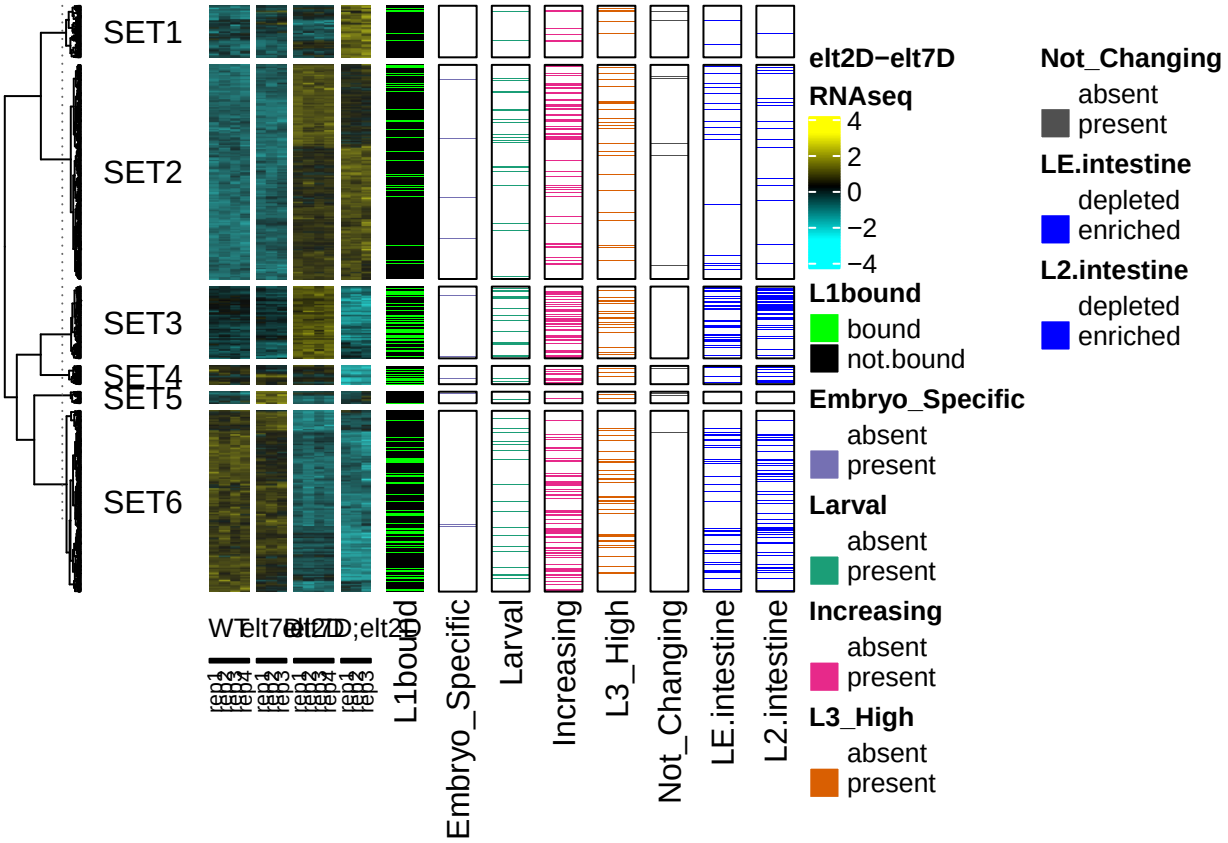



```
if(plot == TRUE){
  myPDFplot(
    plot = Ha_L1chip_clusterchip,
    name = "05a_DE_Heatmap_L1elt2bound_elt2bindclusters_anno",
    height = 6.5,
    width = 6,
    plotdir = plotdir
  )
}
```

Add Spencer intestine RNA row annotation

```
Ha_L1chip_clusterchip_spencerRNA <- Ha_L1chip_clusterchip +
  rowAnnotation(
    LE.intestine = spencer_rna_anno$spencerLE,
    col = list(LE.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  ) +
  rowAnnotation(
    L2.intestine = spencer_rna_anno$spencerL2,
    col = list(L2.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  )
```

```
Ha_L1chip_clusterchip_spencerRNA
```




```
if (plot == TRUE){
  myPDFplot(
    plot = Ha_L1chip_clusterchip_spencerRNA,
    name = "05b_DE_Heatmap_L1elt2bound_elt2bindclusters_spencerRNA_anno",
    height = 6.5,
    width = 8,
    plotdir = plotdir
  )
  }
```

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 = "WBGeneID"
  )

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     absent  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:

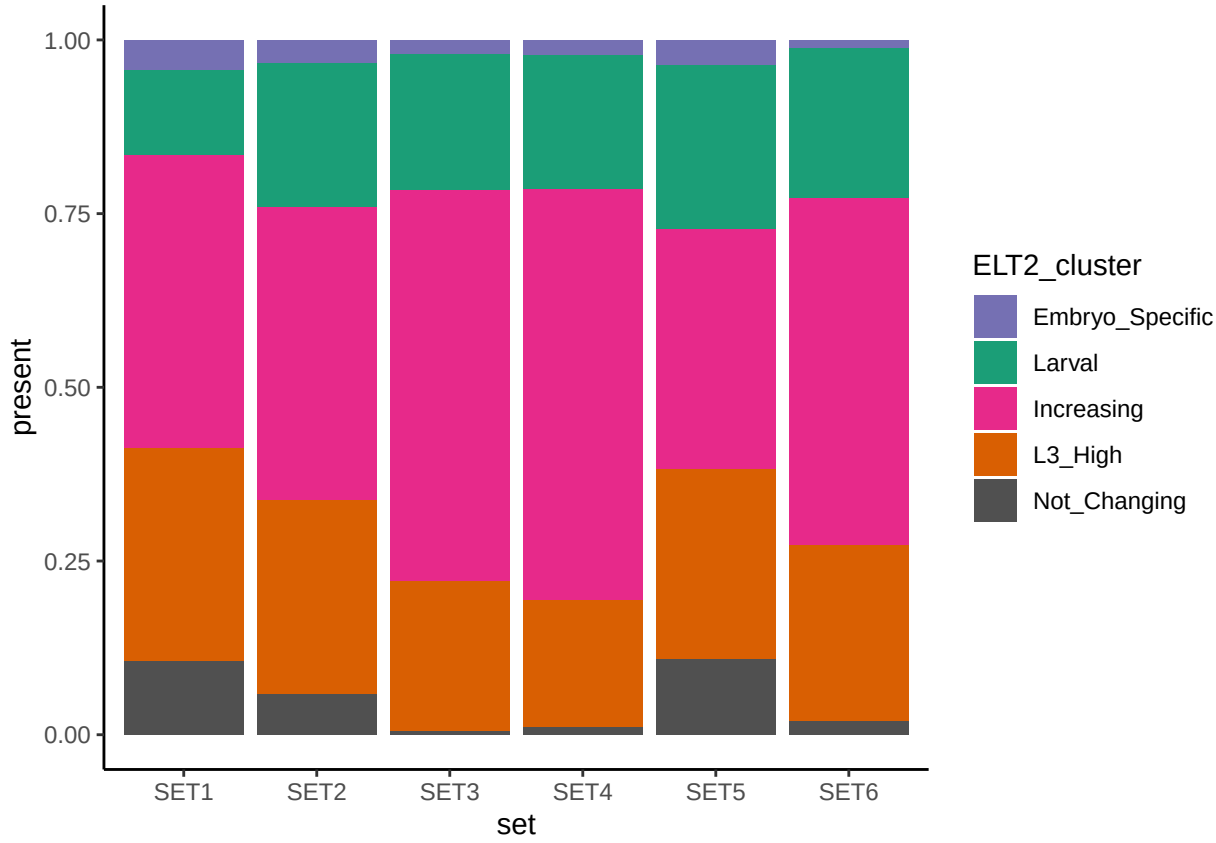
```
expressionSet_per_BindingCluster <- data.frame()
for (i in elt2_cluster_names) {
  toappend1 <-
    table(exprclust_bindclust$set,
          exprclust_bindclust[[i]]) %>%
    as.data.frame.matrix() %>%
    rownames_to_column(var = "set")
    toappend2 <- mutate(toappend1, ELT2_cluster = i,
           percent = present / (present + absent))
  expressionSet_per_BindingCluster <-
    bind_rows(expressionSet_per_BindingCluster, toappend2)
}

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    286       5 Embryo_Specific 0.017182131
## 2  SET2   1188      20 Embryo_Specific 0.016556291
## 3  SET3    397       8 Embryo_Specific 0.019753086
## 4  SET4    101       2 Embryo_Specific 0.019417476
## 5  SET5     63       2 Embryo_Specific 0.030769231
## 6  SET6   1012       8 Embryo_Specific 0.007843137
## 7  SET1    277      14          Larval 0.048109966
## 8  SET2   1087     121          Larval 0.100165563
## 9  SET3    331      74          Larval 0.182716049
## 10 SET4     85      18          Larval 0.174757282
## 11 SET5     52      13          Larval 0.200000000
## 12 SET6    877     143          Larval 0.140196078
## 13 SET1    243      48      Increasing 0.164948454
## 14 SET2    961     247      Increasing 0.204470199
## 15 SET3    191     214      Increasing 0.528395062
## 16 SET4     48      55      Increasing 0.533980583
## 17 SET5     46      19      Increasing 0.292307692
## 18 SET6    688     332      Increasing 0.325490196
## 19 SET1    256      35         L3_High 0.120274914
## 20 SET2   1044     164         L3_High 0.135761589
## 21 SET3    323      82         L3_High 0.202469136
## 22 SET4     86      17         L3_High 0.165048544
## 23 SET5     50      15         L3_High 0.230769231
## 24 SET6    852     168         L3_High 0.164705882
## 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    102       1    Not_Changing 0.009708738
## 29 SET5     59       6    Not_Changing 0.092307692
## 30 SET6   1007      13    Not_Changing 0.012745098
```

Make a plot that addresses the question: What is the percentage of genes with annotated ELT2 binding clusters per expression dataset?

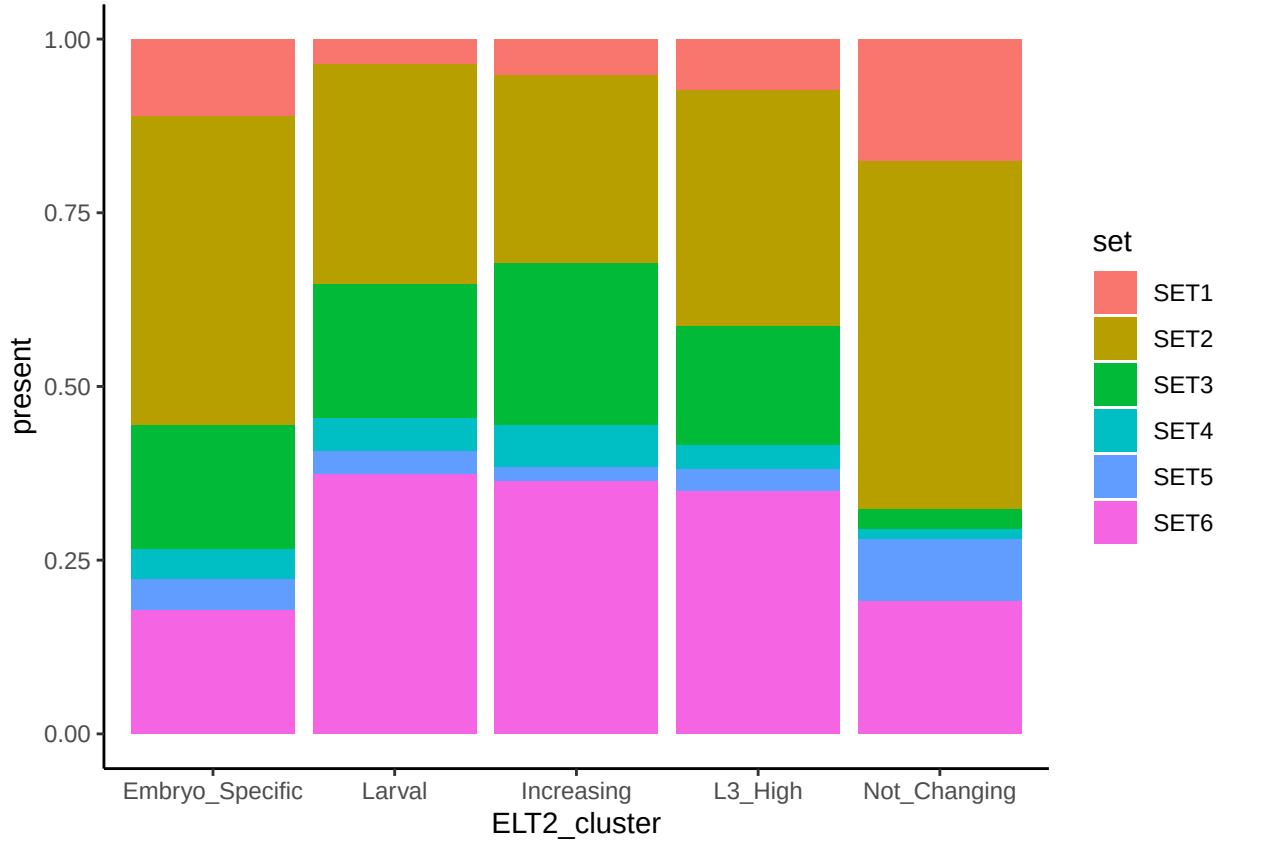
```
percent_bind_cluster_per_DE_set <- ggplot(expressionSet_per_BindingCluster,
       aes(x = set,
           y = present,
           fill = ELT2_cluster)) +
  geom_bar(stat = "identity", position = "fill") +
  theme_classic() +
  scale_fill_manual(values = as.vector(cluster_colors$val))
percent_bind_cluster_per_DE_set
```




```
# ggsave("./03_plots/06_Cluster_percent_present_per_Set_200615.pdf")

if (plot == TRUE){
myggsave(plot = percent_bind_cluster_per_DE_set,
         name = "06_Cluster_percent_present_per_Set",
         plotdir = plotdir,
         width = 5,
         height = 3)
}
```

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

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




```
if (plot == TRUE){
myggsave(plot = expressionSet_per_BindingCluster_plot,
         name = "07_Set_percent_present_per_Cluster",
         plotdir = plotdir,
         width = 5,
         height = 3)
}
```

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

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  0.0172
##  2 SET1  Larval           0.0481
##  3 SET1  Increasing       0.165
##  4 SET1  L3_High          0.120
##  5 SET1  Not_Changing     0.0412
##  6 SET2  Embryo_Specific  0.0166
##  7 SET2  Larval           0.100
##  8 SET2  Increasing       0.204
##  9 SET2  L3_High          0.136
## 10 SET2  Not_Changing     0.0281
## # ... with 20 more rows
```

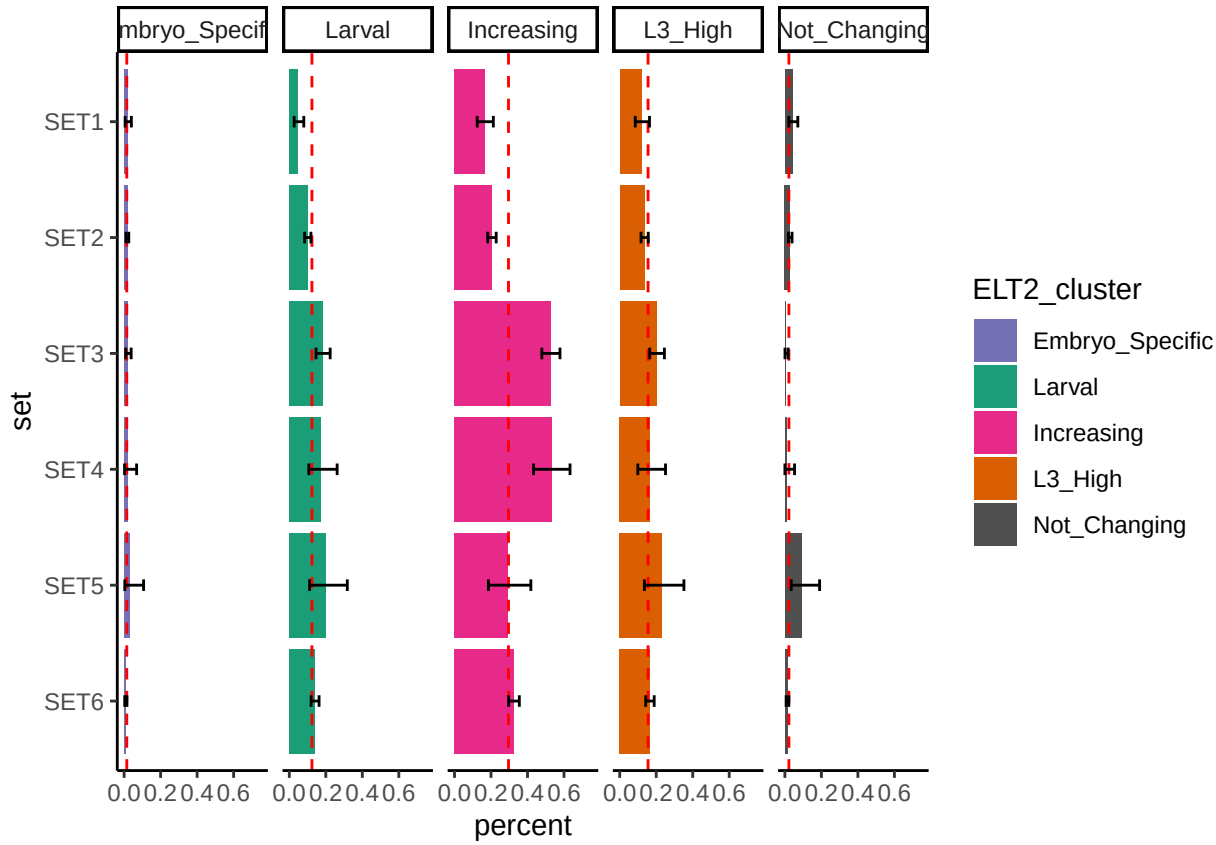
Calculate the binomial pvalue and confidence intervals.

```
# 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     286       5 Embryo_Spec~ 0.0172           0.0146 0.621      0.0396
## 2 SET2    1188      20 Embryo_Spec~ 0.0166           0.0146 0.546      0.0255
## 3 SET3     397       8 Embryo_Spec~ 0.0198           0.0146 0.399      0.0385
## 4 SET4     101       2 Embryo_Spec~ 0.0194           0.0146 0.664      0.0684
## 5 SET5      63       2 Embryo_Spec~ 0.0308           0.0146 0.244      0.107
## 6 SET6    1012       8 Embryo_Spec~ 0.00784          0.0146 0.0869     0.0154
## # ... with 1 more variable: conf.lower <dbl>
```

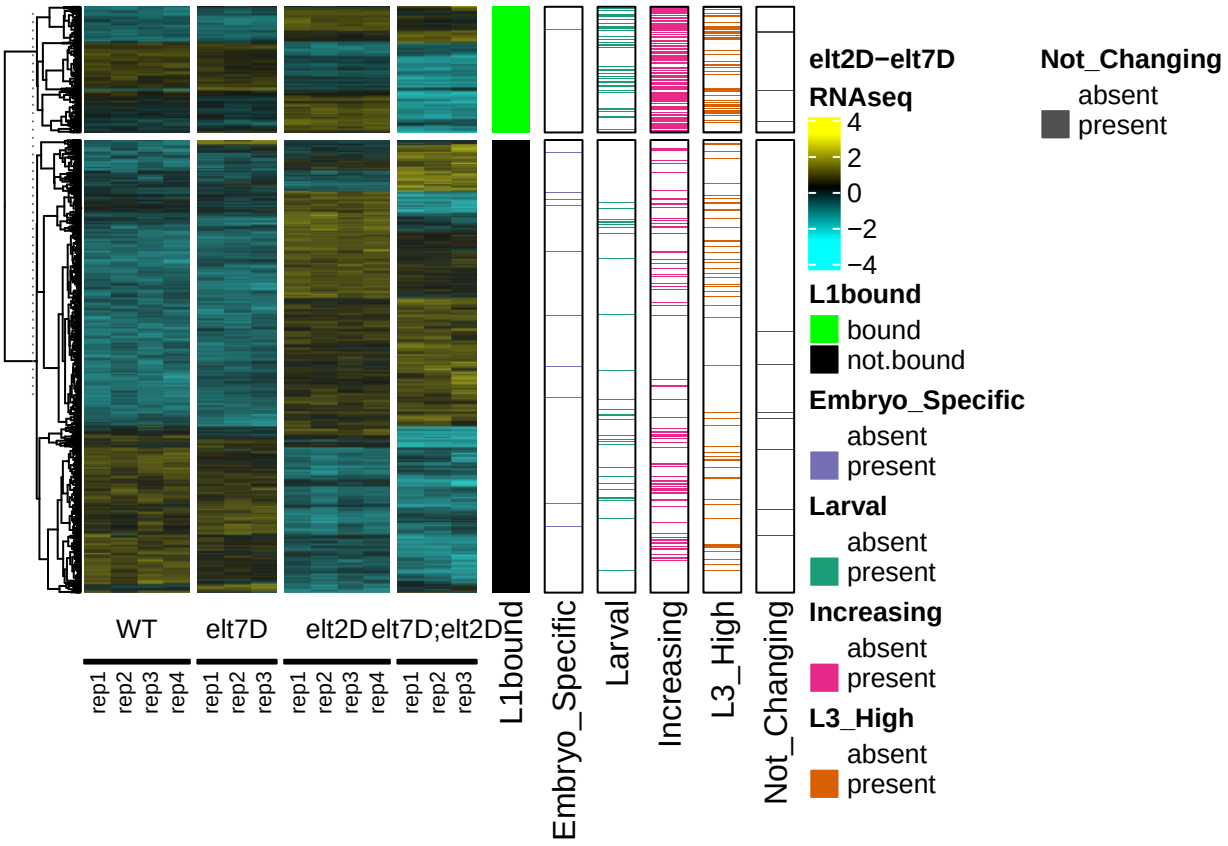
```
per_cluster_pattern_percent <- ggplot(expression_binding_stats,
       aes(x = set,
           y = percent, fill = ELT2_cluster)) +
  geom_bar(stat = "identity") +
  scale_y_continuous(limits = c(0, 0.75)) +
  theme_classic() +
  geom_hline(
    data = percent_bound_per_ELT2_cluster,
    color = "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))

per_cluster_pattern_percent
```




```
if (plot == TRUE){
myggsave(plot = per_cluster_pattern_percent,
      name = "08_Percent_of_ELT2bindClust_per_ExpressionClustf",
       height = 5,
       width = 7,
      plotdir = plotdir)
  }
```

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

```
RNA_heatmap2(
  dynamic_counts_matrix_scaled_ascend,
  column_split = RNA_column_order,
  row_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()
```

```
##         WBGeneID Embryo_Specific  Larval Increasing L3_High Not_Changing
## 1 WBGene00000007          absent  absent    present  absent       absent
## 2 WBGene00000008          absent  absent    present  absent       absent
## 3 WBGene00000064          absent  absent    present present       absent
## 4 WBGene00000067          absent present    present  absent       absent
## 5 WBGene00000107          absent  absent    present present       absent
## 6 WBGene00000136          absent present    present  absent       absent
```

Assign k-means clusters for rows before plotting

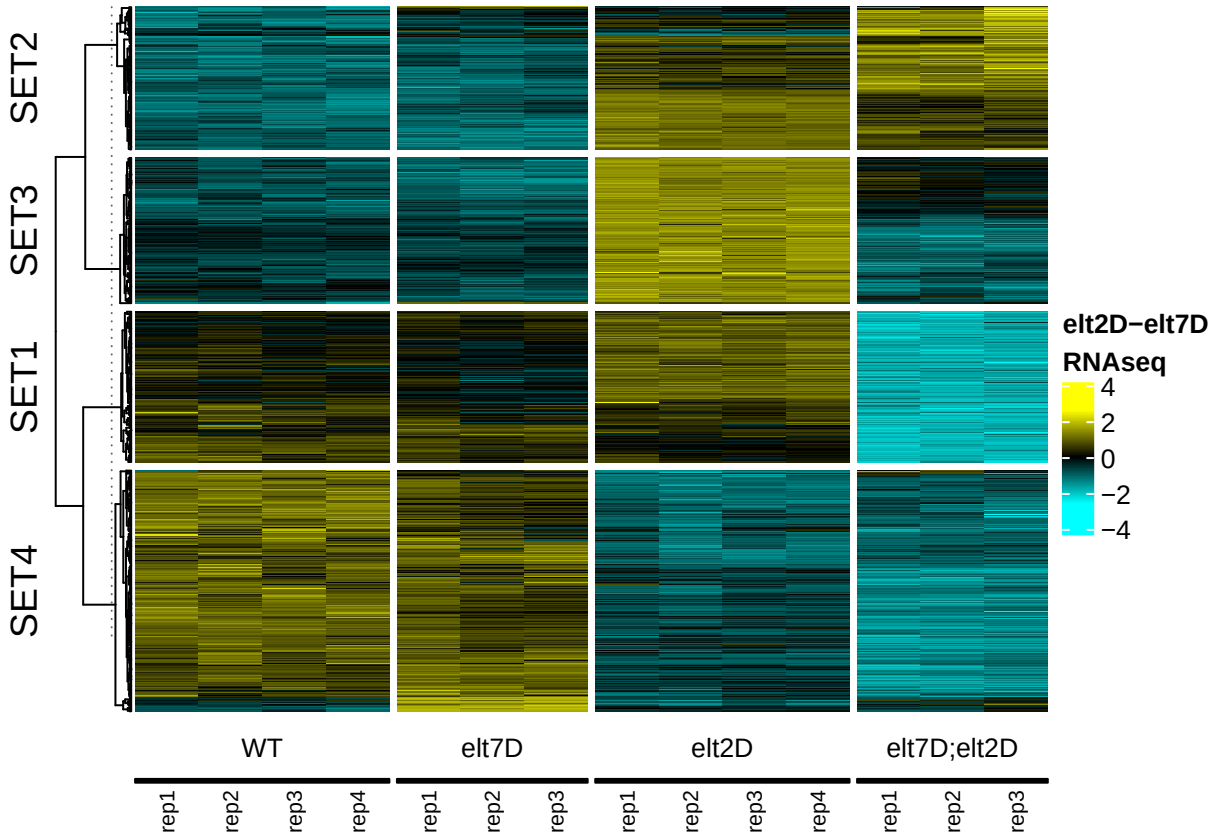
```
kclus <- kmeans(dynamic_counts_matrix_scaled_bound_only, 4)
bound_only_sets <-
```

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

```
##         WBGeneID  set
## 1 WBGene00000007 SET3
## 2 WBGene00000008 SET3
## 3 WBGene00000064 SET1
## 4 WBGene00000067 SET4
## 5 WBGene00000107 SET3
## 6 WBGene00000136 SET3
```

Draw heatmap and check that set assignment is correct.

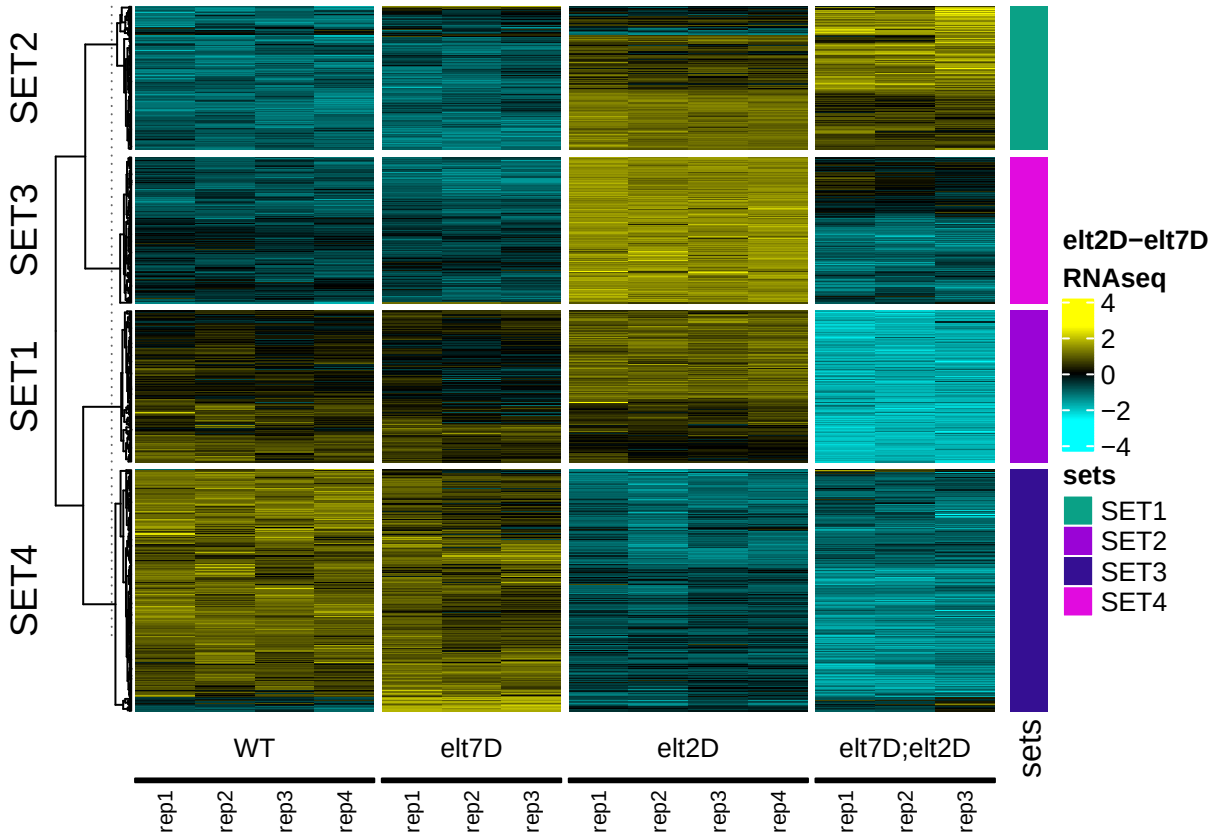
```
Ha_bound_only <-
  RNA_heatmap2(mat = dynamic_counts_matrix_scaled_bound_only,
               column_split = RNA_column_order,
               row_split = bound_only_sets$set,
               row_title = c("SET2", "SET3", "SET1", "SET4"))

Ha_bound_only
```




```
if (plot == TRUE){
  myPDFplot(
    plot = Ha_bound_only,
    name = "09a_DE_Heatmap_L1elt2boundOnly",
```

```
    height = 6.5, width = 6,
    plotdir = plotdir
  )
}
```

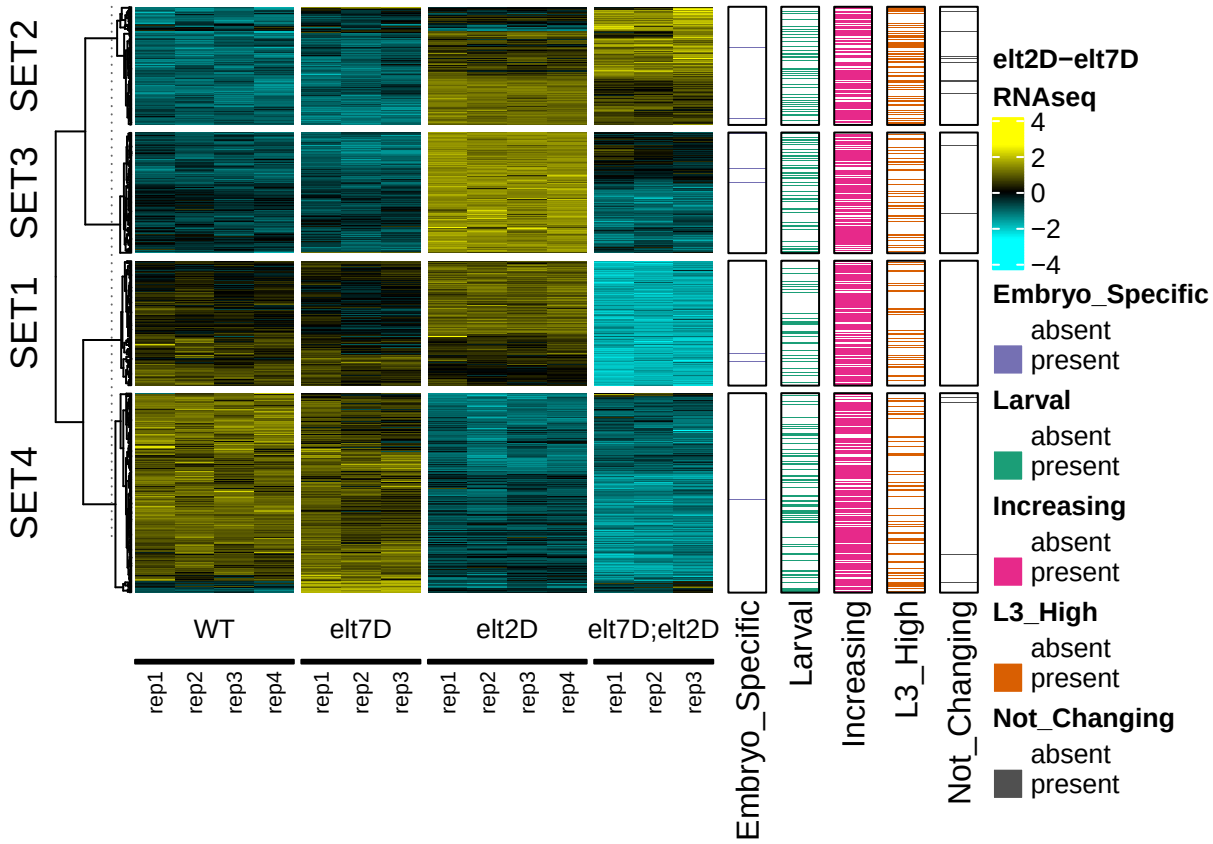
```
Ha_bound_only +
  rowAnnotation(sets = bound_only_sets$set)
```




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

```
##         WBGeneID Embryo_Specific  Larval Increasing L3_High Not_Changing  set
## 1 WBGene00000007          absent  absent    present  absent       absent SET3
## 2 WBGene00000008          absent  absent    present  absent       absent SET3
## 3 WBGene00000064          absent  absent    present present       absent SET1
## 4 WBGene00000067          absent present    present  absent       absent SET4
## 5 WBGene00000107          absent  absent    present present       absent SET3
## 6 WBGene00000136          absent present    present  absent       absent SET3
```

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

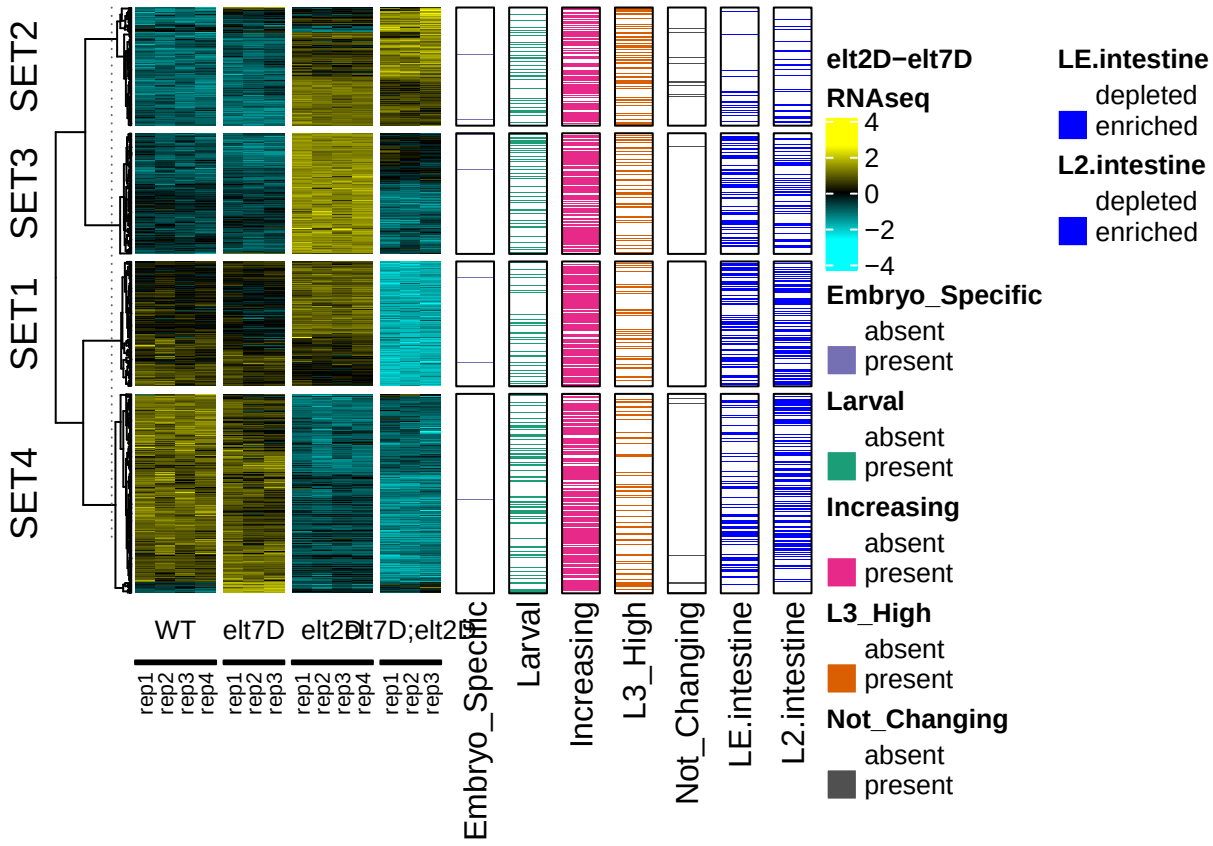



```
if (plot == TRUE){
  myPDFplot(
    plot = Ha_bound_only_chipClust,
    name = "09b_DE_Heatmap_L1elt2boundOnly_elt2bindclusters_anno",
    height = 6.5,
    width = 6,
    plotdir = plotdir
  )
  }
```

Add Spencer intestine expression row annotation

```
bound_only_spencer_rna_anno <- data.frame(
  spencerLE = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_bound_only) %in% spencer_LE_subset$spencer_LE_ID,
    yes = "enriched",
    no = "depleted"
  ),
  spencerL2 = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_bound_only) %in% spencer_L2_subset$spencer_L2_ID,
    yes = "enriched",
    no = "depleted"
  )
)

Ha_bound_only_chipClust_spencer <- Ha_bound_only_chipClust +
```

```
  rowAnnotation(
    LE.intestine = bound_only_spencer_rna_anno$spencerLE,
    col = list(LE.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  ) +
  rowAnnotation(
    L2.intestine = bound_only_spencer_rna_anno$spencerL2,
    col = list(L2.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  )

Ha_bound_only_chipClust_spencer
```




```
if (plot == TRUE){
  myPDFplot(
    plot = Ha_bound_only_chipClust_spencer,
    name = "09c_DE_Heatmap_L1elt2boundOnly_elt2bindclusters_spencerRNA",
    height = 6.5,
    width = 6,
    plotdir = plotdir
  )
  }
```

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

```
bound_only_exprclust_bindclust <-
  merge(bound_only_sets,
        chip_annotation_present_absent,
        by.x = "WBGeneID",
        by.y = "WBGeneID")

bound_only_exprclust_bindclust %>% head
```

```
##         WBGeneID  set Embryo_Specific  Larval Increasing L3_High Not_Changing
## 1 WBGene00000007 SET3          absent  absent    present  absent       absent
## 2 WBGene00000008 SET3          absent  absent    present  absent       absent
## 3 WBGene00000064 SET1          absent  absent    present present       absent
## 4 WBGene00000067 SET4          absent present    present  absent       absent
## 5 WBGene00000107 SET3          absent  absent    present present       absent
## 6 WBGene00000136 SET3          absent present    present  absent       absent
```

Make a dataframe that addresses the question:

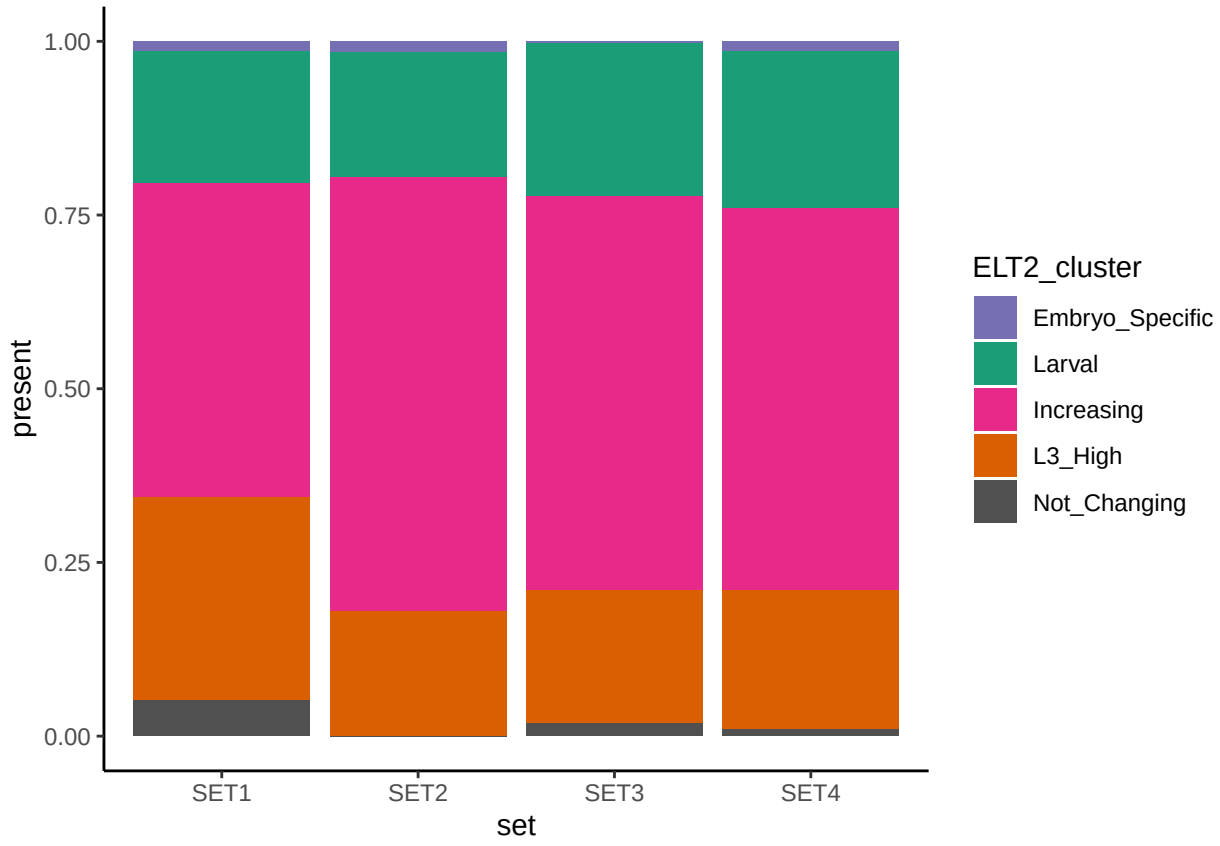
```
bound_only_expressionSet_per_BindingCluster <- data.frame()
for (i in elt2_cluster_names) {
  toappend <-
    table(bound_only_exprclust_bindclust$set,
          bound_only_exprclust_bindclust[[i]]) %>%
    as.data.frame.matrix() %>%
    rownames_to_column(var = "set") %>%
    mutate(ELT2_cluster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

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

bound_only_expressionSet_per_BindingCluster
```

```
##     set absent present    ELT2_cluster     percent
## 1  SET1    138       3 Embryo_Specific 0.021276596
## 2  SET2    146       3 Embryo_Specific 0.020134228
## 3  SET3    237       1 Embryo_Specific 0.004201681
## 4  SET4    141       3 Embryo_Specific 0.020833333
## 5  SET1    100      41          Larval 0.290780142
## 6  SET2    114      35          Larval 0.234899329
## 7  SET3    167      71          Larval 0.298319328
## 8  SET4     98      46          Larval 0.319444444
## 9  SET1     44      97      Increasing 0.687943262
## 10 SET2     27     122      Increasing 0.818791946
## 11 SET3     55     183      Increasing 0.768907563
## 12 SET4     32     112      Increasing 0.777777778
## 13 SET1     78      63         L3_High 0.446808511
## 14 SET2    114      35         L3_High 0.234899329
```

```
## 15 SET3    176      62         L3_High 0.260504202
## 16 SET4    103      41         L3_High 0.284722222
## 17 SET1    130      11    Not_Changing 0.078014184
## 18 SET2    149       0    Not_Changing 0.000000000
## 19 SET3    232       6    Not_Changing 0.025210084
## 20 SET4    142       2    Not_Changing 0.013888889
```

Make a plot that addresses the question: What is the percentage of genes with annotated ELT2 binding clusters per expression dataset?

```
bound_percent_plot <- ggplot(
  bound_only_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))

bound_percent_plot
```

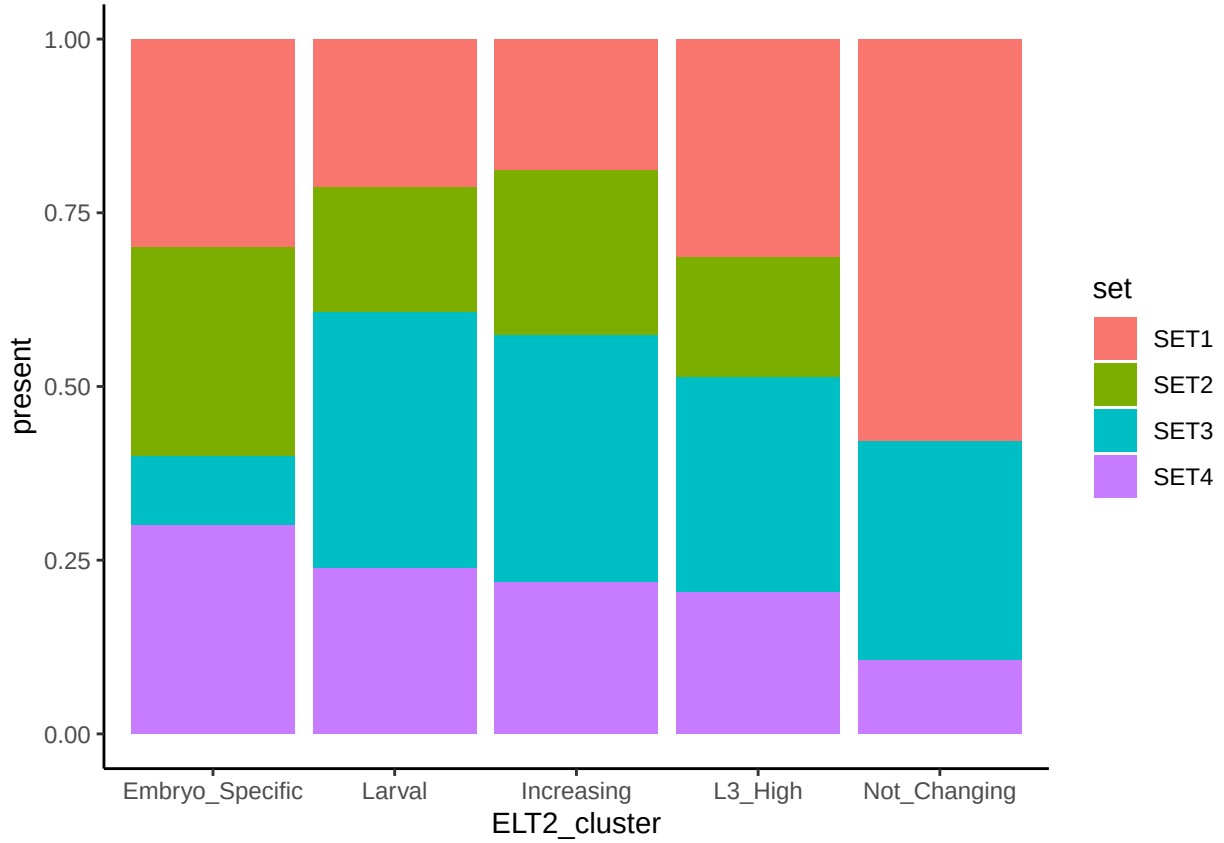



```
if (plot == TRUE){
myggsave(plot = bound_percent_plot,
         name = "10a_Bound_Only_Cluster_percent_present_per_Set",
         height = 3,
         width = 5,
         plotdir = plotdir)
```

```
}
```

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

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




```
if (plot == TRUE){
myggsave(plot = bound_percent_plot_inverse,
         name = "10b_Bound_Only_Set_percent_present_per_Cluster",
         height = 3,
         width = 5,
         plotdir = plotdir)
}
```

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

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

```
bound_only_percent_bound_per_ELT2_cluster <-
  bound_only_expressionSet_per_BindingCluster %>% group_by(ELT2_cluster) %>% summarise(percent = sum(pr
```

```
                                                                    nrow(dynamic_counts_matri
```

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

```
bound_only_expressionSet_per_BindingCluster %>% group_by(set, ELT2_cluster) %>% summarise(percent = pres
                                                                                  (present + absent))
```

```
## # A tibble: 20 x 3
## # Groups:   set [4]
##    set   ELT2_cluster    percent
##    <chr> <fct>            <dbl>
##  1 SET1  Embryo_Specific 0.0213
##  2 SET1  Larval          0.291
##  3 SET1  Increasing      0.688
##  4 SET1  L3_High         0.447
##  5 SET1  Not_Changing    0.0780
##  6 SET2  Embryo_Specific 0.0201
##  7 SET2  Larval          0.235
##  8 SET2  Increasing      0.819
##  9 SET2  L3_High         0.235
## 10 SET2  Not_Changing    0
## 11 SET3  Embryo_Specific 0.00420
## 12 SET3  Larval          0.298
## 13 SET3  Increasing      0.769
## 14 SET3  L3_High         0.261
## 15 SET3  Not_Changing    0.0252
## 16 SET4  Embryo_Specific 0.0208
## 17 SET4  Larval          0.319
## 18 SET4  Increasing      0.778
## 19 SET4  L3_High         0.285
## 20 SET4  Not_Changing    0.0139
```

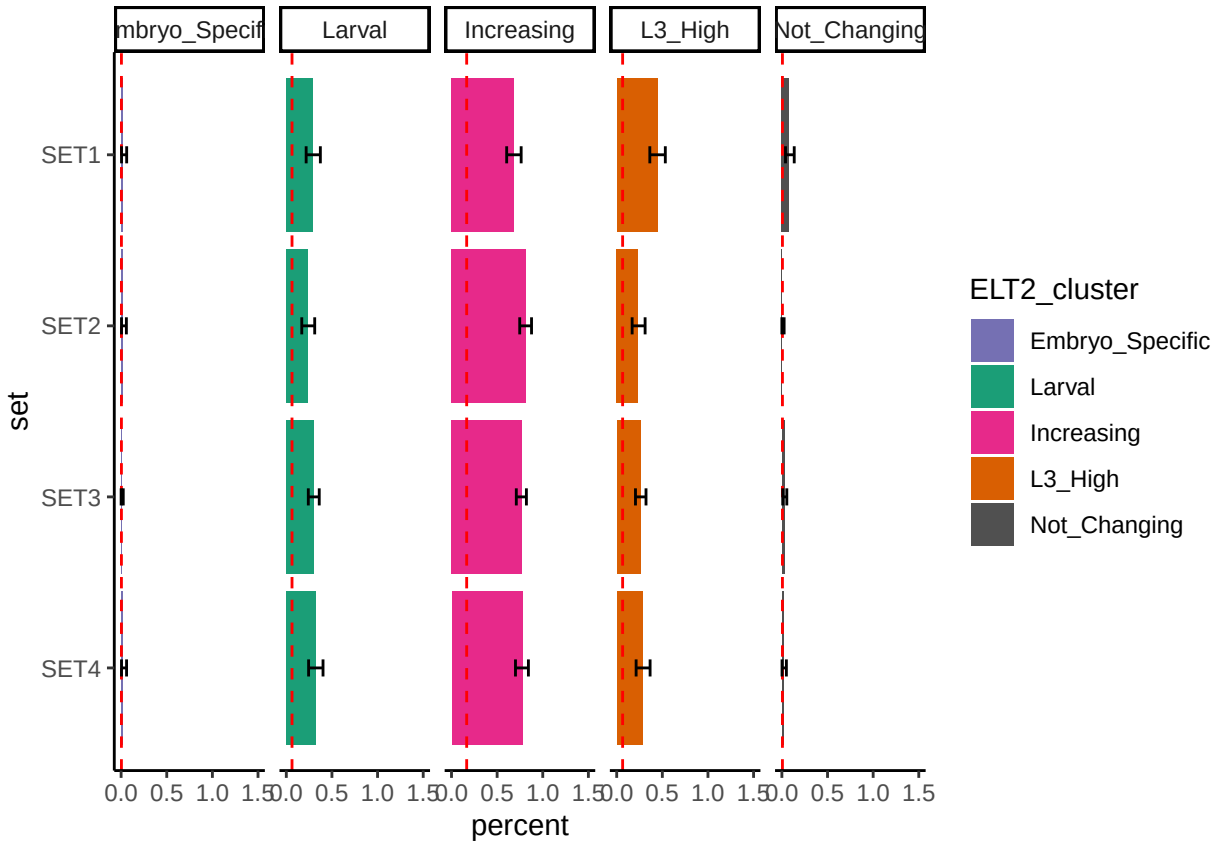
Calculate the binomial pvalue and confidence intervals.

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

# Use binom.test to calculate pvalue and confidence intervales for the percentage of ELT2 binding clust
bound_only_expression_binding_stats <-
  bound_only_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]
  )

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

bound_only_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     138       3 Embryo_Spec~ 0.0213           0.0149 0.471     0.0609
## 2 SET2     146       3 Embryo_Spec~ 0.0201           0.0149 0.489     0.0577
## 3 SET3     237       1 Embryo_Spec~ 0.00420          0.0149 0.276     0.0232
## 4 SET4     141       3 Embryo_Spec~ 0.0208           0.0149 0.478     0.0597
## 5 SET1     100      41 Larval       0.291            0.287  0.926     0.373
## 6 SET2     114      35 Larval       0.235            0.287  0.175     0.311
## # ... with 1 more variable: conf.lower <dbl>
```

```
bound_percent_multi <- ggplot(bound_only_expression_binding_stats,
       aes(x = set,
           y = percent, fill = ELT2_cluster)) +
  geom_bar(stat = "identity") +
  scale_y_continuous(limits = c(0, 1.5)) +
  theme_classic() +
  geom_hline(
    data = bound_only_percent_bound_per_ELT2_cluster,
    color = "red",
    linetype = "dashed",
    aes(yintercept = percent)
  ) +
  geom_errorbar(
    ymax = bound_only_expression_binding_stats$conf.upper,
    ymin = bound_only_expression_binding_stats$conf.lower,
    width = 0.1
  ) +
  coord_flip() +
  facet_grid(. ~ ELT2_cluster) +
  scale_fill_manual(values = as.character(cluster_colors$val))

bound_percent_multi
```

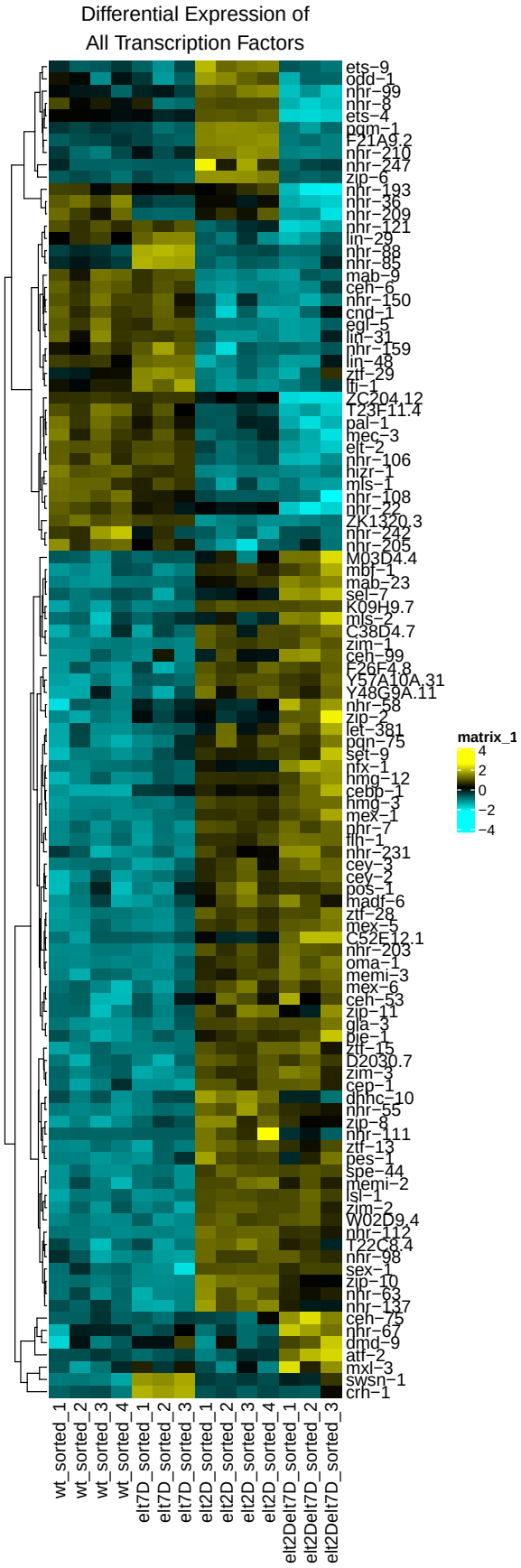



```
if (plot == TRUE){
myggsave(plot = bound_percent_multi,
         name = "11_Bound_Only_Percent_of_ELT2bindClust_per_ExpressionClust",
         height = 5,
         width = 8,
         plotdir = plotdir)
}
```

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\nAll Transcription Factors"
)
tf_heatmap
```


Differential 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
lfi−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
cebb−1
hmg−3
mex−1
phr−7
tlh−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
dnhc−10
nhr−55
zip−8
nhr−111
ztf−13
pes−1
spe−44
memi−2
lsl−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
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
matrix_1
4
2
0
−2
−4

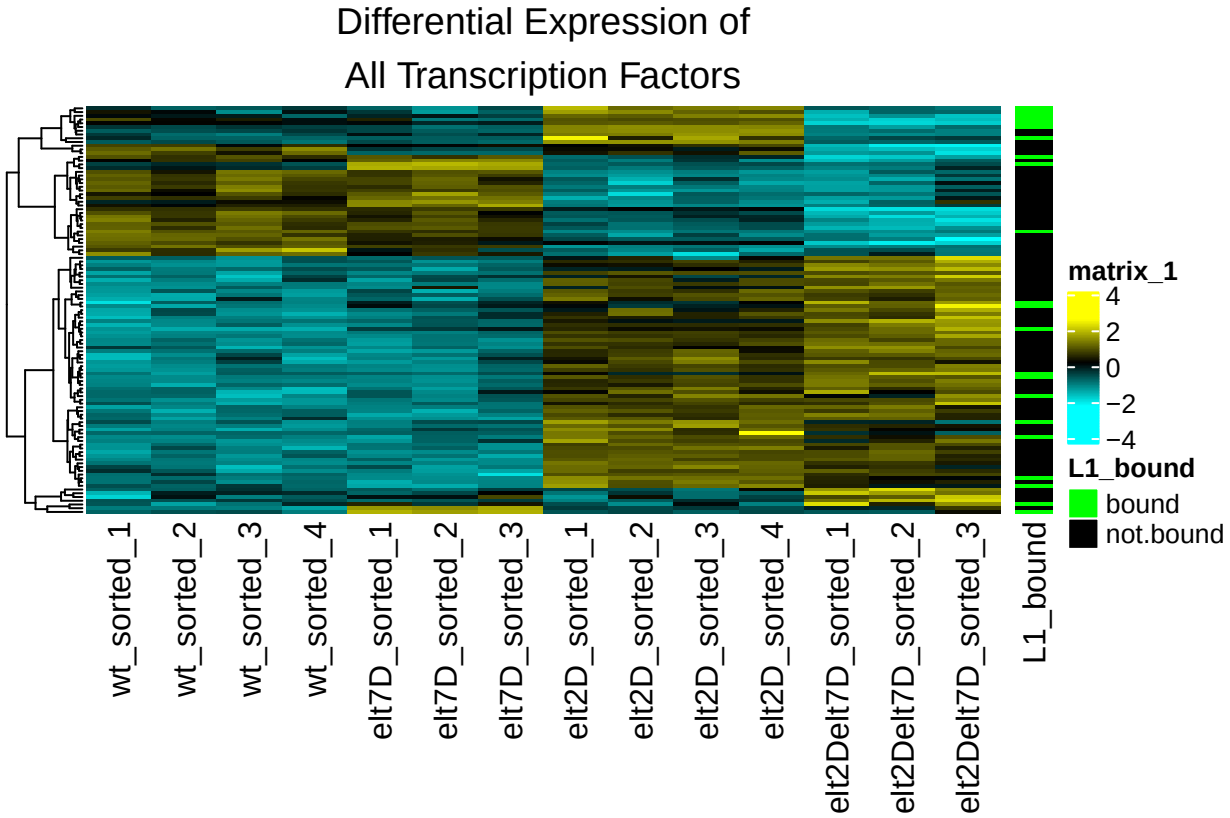
```
if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap,
    name = "12_Differential_Expression_of_All_TFs",
    height = 20,
    width = 4,
    plotdir = plotdir
  )
  }
```

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: 22 x 1
##    WBGeneID
##    <chr>
##  1 WBGene00004096
##  2 WBGene00019327
##  3 WBGene00003711
##  4 WBGene00000793
##  5 WBGene00021082
##  6 WBGene00003607
##  7 WBGene00019743
##  8 WBGene00003689
##  9 WBGene00003648
## 10 WBGene00012101
## # ... with 12 more rows
```

```
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
```

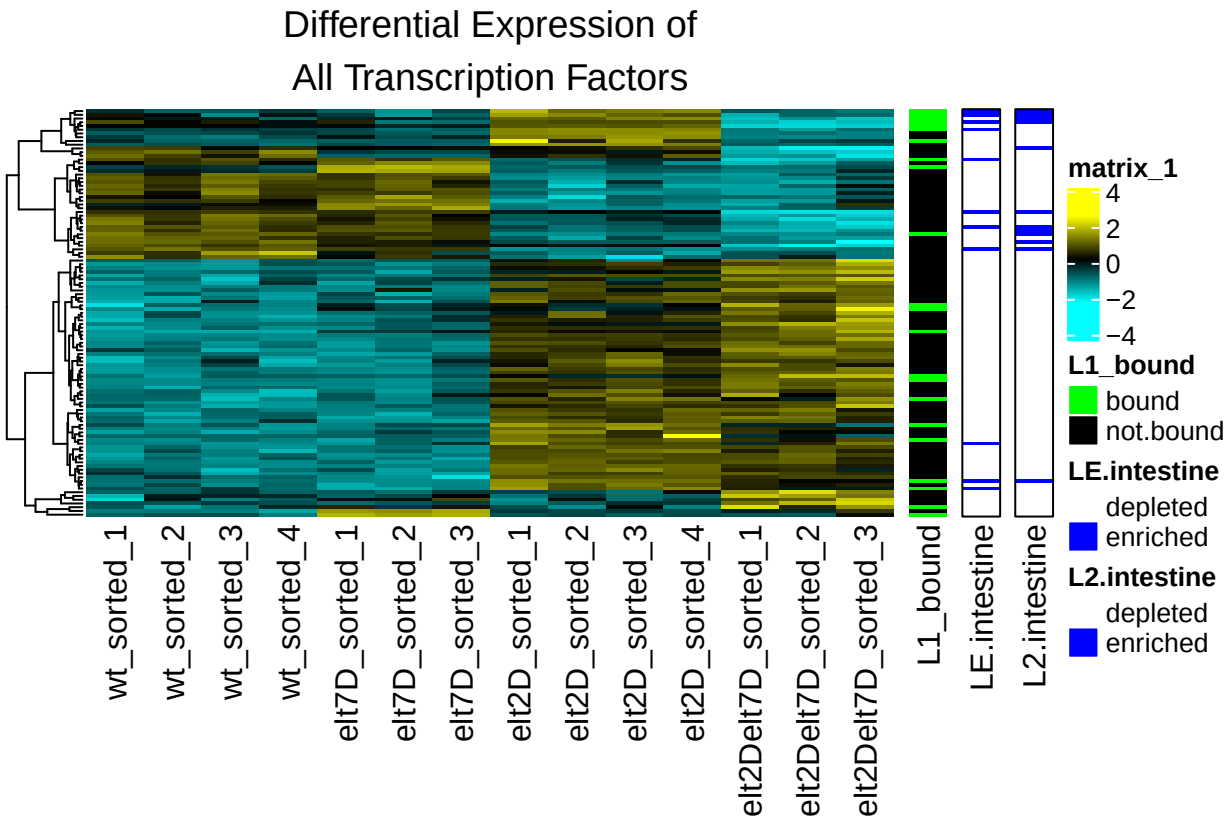



```
# pdf("./03_plots/13a_Differential_Expression_of_All_TFs_L1elt2bound_anno.pdf", height = 5, width = 5.5
# tf_heatmap_L1bound
# dev.off()

if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap_L1bound,
    name = "13a_Differential_Expression_of_All_TFs_L1elt2bound_anno",
    height = 5,
    width = 5.5,
    plotdir = plotdir
  )
  }
```

Add row annotation of intestine expression from Spencer intestine RNA data

```
tf_spencer_rna_anno <- data.frame(
  spencerLE = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_TFs) %in% spencer_LE_subset$spencer_LE_ID,
    yes = "enriched",
    no = "depleted"
  ),
  spencerL2 = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_TFs) %in% spencer_L2_subset$spencer_L2_ID,
    yes = "enriched",
    no = "depleted"
  )
)
```

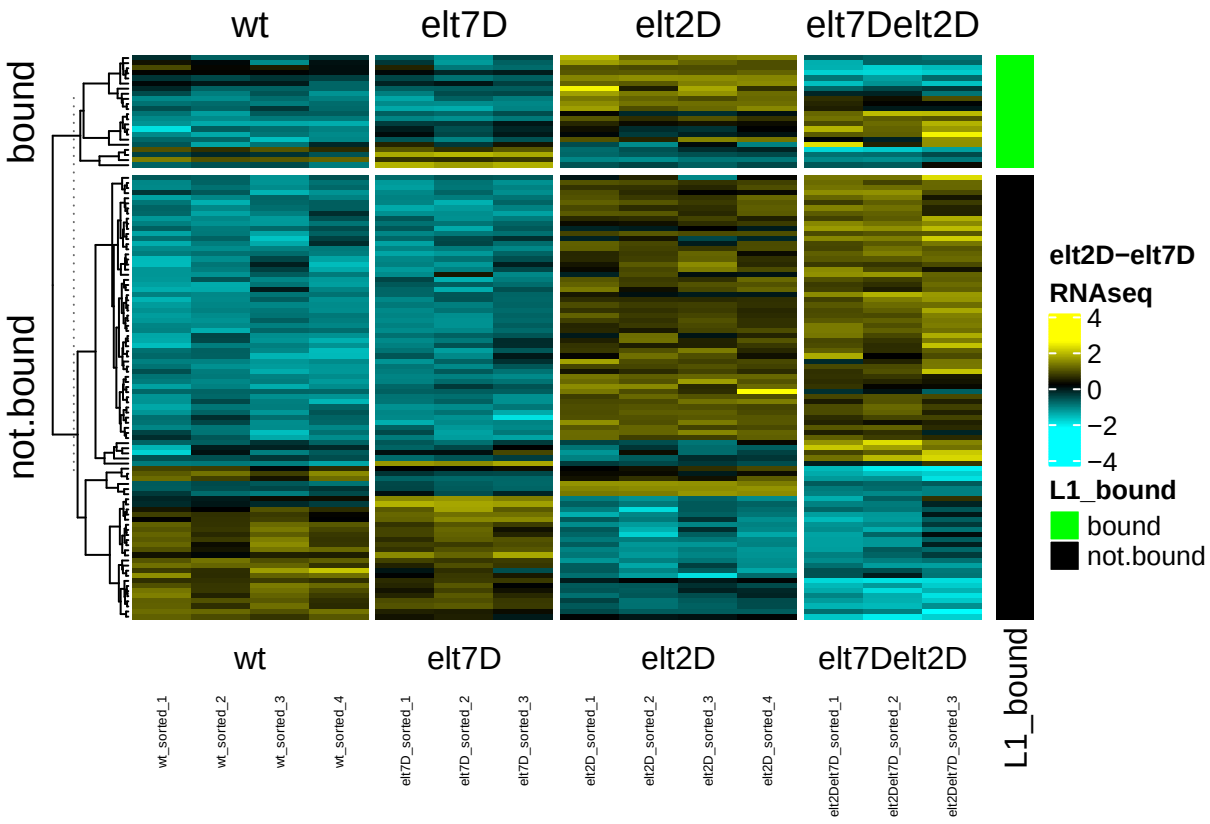
```
tf_heatmap_L1bound_spencerRNA <- tf_heatmap_L1bound + rowAnnotation(
    LE.intestine = tf_spencer_rna_anno$spencerLE,
    col = list(LE.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  ) +
  rowAnnotation(
    L2.intestine = tf_spencer_rna_anno$spencerL2,
    col = list(L2.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  )

tf_heatmap_L1bound_spencerRNA
```




```
if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap_L1bound_spencerRNA,
    name = "13b_Differential_Expression_of_All_TFs_L1elt2bound_anno",
    height = 5,
    width = 5.5,
    plotdir = plotdir
  )
  }
```

Split heatmap based on L1 binding

```
tf_heatmap_L1bound_split <- 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"
                )))
tf_heatmap_L1bound_split
```

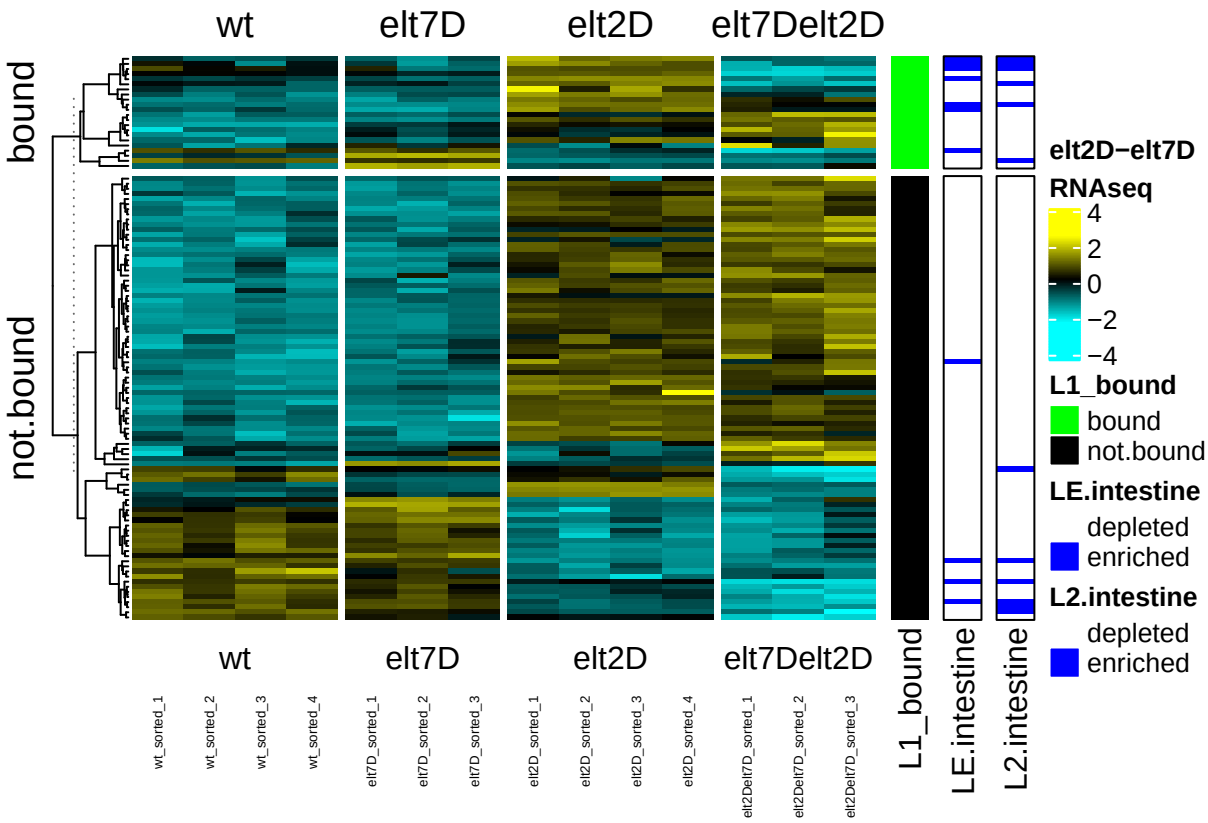



```
if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap_L1bound_split,
    name = "14a_Differential_Expression_of_All_TFs_L1elt2bound_split",
    height = 5,
    width = 5.5,
    plotdir = plotdir
  )
  }
```

Add row annotation of intestine expression from Spencer intestine RNA data to split heatmap

```
tf_heatmap_L1bound_split_spencerRNA <- tf_heatmap_L1bound_split +
  rowAnnotation(
    LE.intestine = tf_spencer_rna_anno$spencerLE,
    col = list(LE.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  ) +
```

```
  rowAnnotation(
    L2.intestine = tf_spencer_rna_anno$spencerL2,
    col = list(L2.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  )

tf_heatmap_L1bound_split_spencerRNA
```

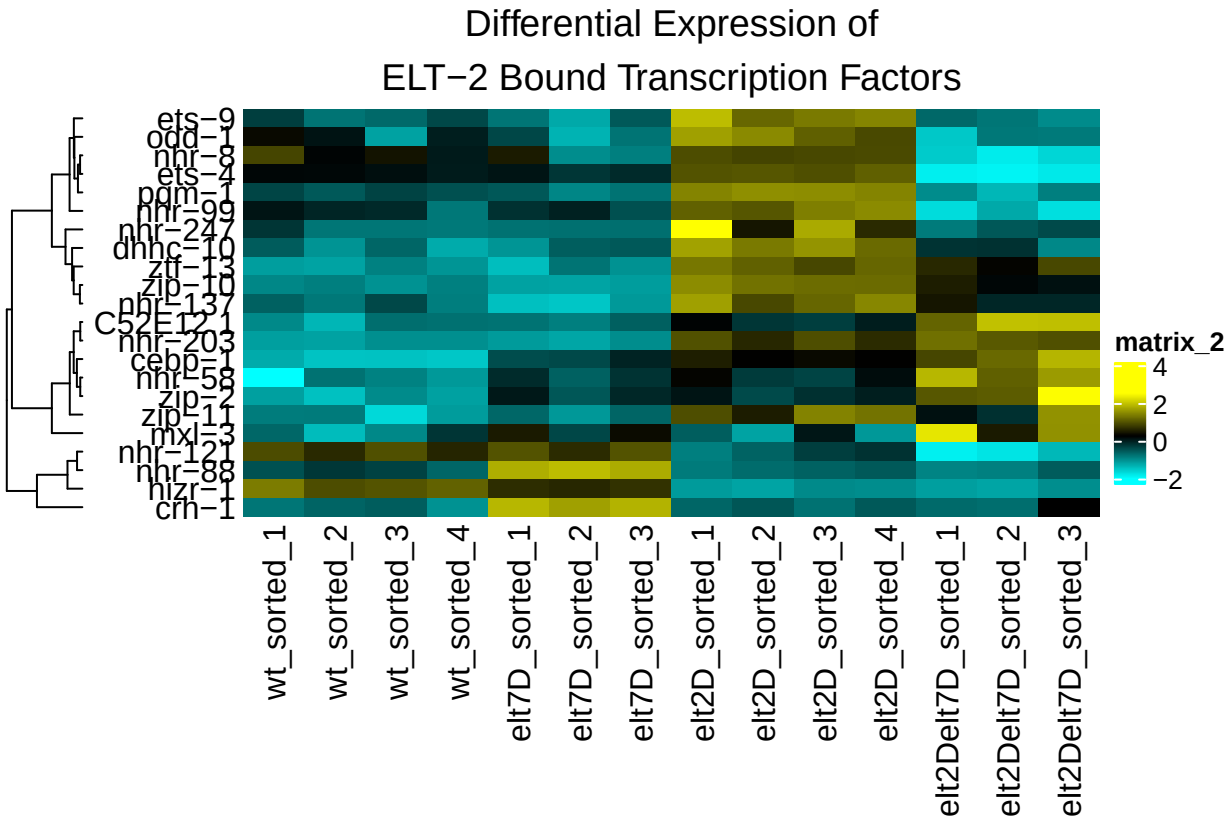



```
if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap_L1bound_split_spencerRNA,
    name = "14b_Differential_Expression_of_All_TFs_L1elt2bound_split_spencerRNA",
    height = 5,
    width = 5.5,
    plotdir = plotdir
  )
  }
```

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)
```

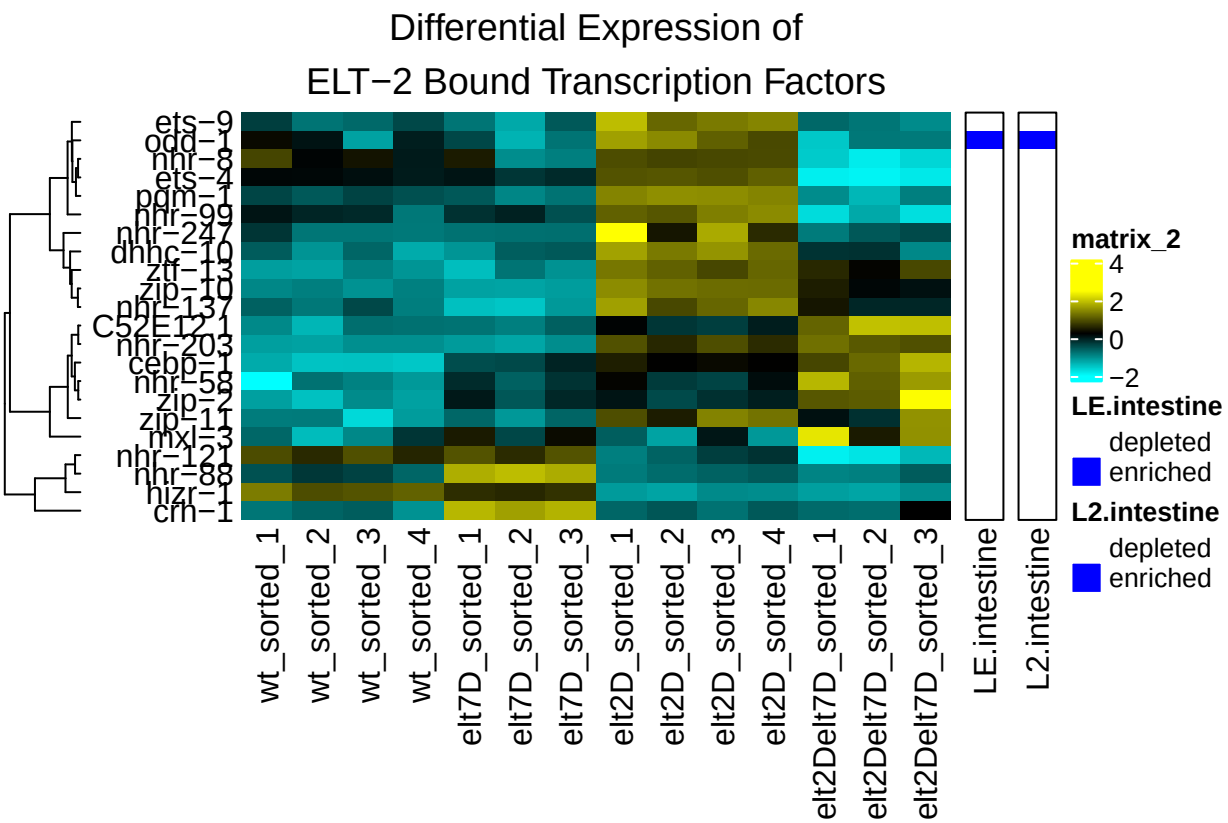
```
HAboundTF <- Heatmap(
  dynamic_counts_matrix_scaled_TFs_bound_names,
  col = colorRampPalette(c("cyan", "black", "yellow"))(1000),
  cluster_columns = FALSE,
  clustering_distance_rows = "spearman",
  clustering_method_rows = "complete",
  show_row_names = TRUE,
  row_names_side = "left",
  show_column_names = TRUE,
  column_title = "Differential Expression of\nELT-2 Bound Transcription Factors"
)

HAboundTF
```




```
if (plot == TRUE){
  myPDFplot(
    plot = HAboundTF,
    name = "15a_Differential_Expression_Bound_TFs_only",
    height = 5,
    width = 5.5,
    plotdir = plotdir
  )
  }
```

```
tf_bound_spencer_rna_anno <- data.frame(
  spencerLE = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_TFs_bound) %in% spencer_LE_subset$spencer_LE_ID,
    yes = "enriched",
```

```
    no = "depleted"
  ),
  spencerL2 = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_TFs_bound) %in% spencer_L2_subset$spencer_L2_ID,
    yes = "enriched",
    no = "depleted"
  )
)

HAboundTF_spencerRNA <- HAboundTF + rowAnnotation(
    LE.intestine = tf_spencer_rna_anno$spencerLE,
    col = list(LE.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  ) +
  rowAnnotation(
    L2.intestine = tf_spencer_rna_anno$spencerL2,
    col = list(L2.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  )

HAboundTF_spencerRNA
```




```
if (plot == TRUE){
  myPDFplot(
```

```
    plot = HAboundTF_spencerRNA,
    name = "15b_Differential_Expression_Bound_TFs_only_spencerRNA",
    height = 5,
    width = 5.5,
    plotdir = plotdir
  )
  }
```

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

TFs to follow up: pqm-1 (intestine), 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

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] lubridate_1.7.8      circlize_0.4.9       binom_1.1-1
##  [4] dendextend_1.13.4    RVAideMemoire_0.9-75 pheatmap_1.0.12
##  [7] matrixStats_0.56.0   ComplexHeatmap_2.2.0 readxl_1.3.1
## [10] forcats_0.5.0        stringr_1.4.0        dplyr_0.8.5
## [13] purrr_0.3.4          readr_1.3.1          tidyr_1.0.3
## [16] tibble_3.0.1         ggplot2_3.3.0        tidyverse_1.3.0
## [19] biomaRt_2.42.1
##
## loaded via a namespace (and not attached):
##  [1] nlme_3.1-147         fs_1.4.1             bit64_0.9-7
##  [4] RColorBrewer_1.1-2   progress_1.2.2       httr_1.4.1
##  [7] tools_3.6.3          backports_1.1.6      utf8_1.1.4
## [10] R6_2.4.1             DBI_1.1.0            BiocGenerics_0.32.0
## [13] colorspace_1.4-1     GetoptLong_0.1.8     withr_2.2.0
## [16] gridExtra_2.3        tidyselect_1.0.0     prettyunits_1.1.1
## [19] bit_1.1-15.2         curl_4.3             compiler_3.6.3
## [22] cli_2.0.2            rvest_0.3.5          Biobase_2.46.0
## [25] xml2_1.3.2           labeling_0.3         scales_1.1.0
## [28] askpass_1.1          rappdirs_0.3.1       digest_0.6.25
## [31] rmarkdown_2.1        pkgconfig_2.0.3      htmltools_0.4.0
```

```
## [34] dbplyr_1.4.3         rlang_0.4.6          GlobalOptions_0.1.1
## [37] rstudioapi_0.11      RSQLite_2.2.0        farver_2.0.3
## [40] shape_1.4.4          generics_0.0.2       jsonlite_1.6.1
## [43] magrittr_1.5         Rcpp_1.0.4.6         munsell_0.5.0
## [46] S4Vectors_0.24.4     fansi_0.4.1          viridis_0.5.1
## [49] lifecycle_0.2.0      stringi_1.4.6        yaml_2.2.1
## [52] BiocFileCache_1.10.2 blob_1.2.1           parallel_3.6.3
## [55] crayon_1.3.4         lattice_0.20-41      haven_2.2.0
## [58] hms_0.5.3            knitr_1.28           pillar_1.4.4
## [61] rjson_0.2.20         stats4_3.6.3         reprex_0.3.0
## [64] XML_3.99-0.3         glue_1.4.0           evaluate_0.14
## [67] modelr_0.1.7         png_0.1-7            vctrs_0.2.4
## [70] cellranger_1.1.0     gtable_0.3.0         openssl_1.4.1
## [73] clue_0.3-57          assertthat_0.2.1     xfun_0.13
## [76] broom_0.5.6          viridisLite_0.3.0    AnnotationDbi_1.48.0
## [79] memoise_1.1.0        IRanges_2.20.2       cluster_2.1.0
## [82] ellipsis_0.3.0
```