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 <- TRUE
plotdir <- "./03_plots/"</pre>
```

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 -----
                                       ----- tidyverse 1.3.0 --
## v ggplot2 3.3.0
                            0.3.4
                   v purrr
## 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 ----- tidyverse_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

- 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

2 WBGene00015956

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
## WBGene0000001
                     8.957161
                                  8.858238
                                              8.841623
                                                          8.923111
                                                                          8.505028
## WBGene00000002
                     7.489159
                                  7.382905
                                              7.518631
                                                          7.492399
                                                                          7.378168
## WBGene0000003
                     9.061810
                                 8.748589
                                              9.295497
                                                          9.286834
                                                                          9.480361
## WBGene0000004
                    10.916559
                                10.786200
                                             11.010430
                                                          10.826657
                                                                         10.836827
## WBGene0000005
                                              3.116144
                     2.990777
                                  2.864044
                                                          2.715502
                                                                          2.584081
## WBGene0000007
                     5.799066
                                  6.026780
                                              5.831420
                                                          6.072836
                                                                          5.699261
##
                  elt7D_sorted_2 elt7D_sorted_3 elt2D_sorted_1 elt2D_sorted_2
## WBGene0000001
                        8.568569
                                        8.517438
                                                       9.172904
                                                                       9.249496
## WBGene00000002
                                                                       7.289884
                        7.582425
                                        7.512668
                                                       7.503760
## WBGene0000003
                        9.451384
                                        9.008938
                                                       8.669299
                                                                       8.593847
## WBGene0000004
                       10.806534
                                       10.819497
                                                      10.303062
                                                                      10.296768
## WBGene0000005
                        2.881642
                                        2.827526
                                                       2.953325
                                                                       2.835451
## WBGene0000007
                        5.492677
                                        5.220378
                                                       4.683237
                                                                       4.797660
                  elt2D_sorted_3 elt2D_sorted_4 elt2Delt7D_sorted_1
## WBGene0000001
                        9.211660
                                        9.346959
                                                             9.379698
## WBGene00000002
                        7.386127
                                        7.262063
                                                             7.904008
## WBGene0000003
                        8.753835
                                        8.781267
                                                             8.791018
## WBGene00000004
                                                            10.332489
                       10.356820
                                       10.366512
## WBGene0000005
                        2.886842
                                        2.979650
                                                             2.499412
## WBGene00000007
                                                             4.602235
                        4.495252
                                        4.593047
##
                  elt2Delt7D sorted 2 elt2Delt7D sorted 3
## WBGene0000001
                             9.217403
                                                  9.101997
## WBGene00000002
                             7.870852
                                                  7.762023
## WBGene0000003
                             8.795191
                                                  8.936724
## WBGene0000004
                             10.223675
                                                 10.597407
## WBGene0000005
                              2.763405
                                                  2.428255
## WBGene0000007
                             4.641832
                                                  4.476899
list of all dynamically expressed genes
dynamic_regulated_genes <-</pre>
  read.table(file = "./01_input/2017-11-20_all_changing_genes_0.1alpha_0.8lfc.txt",
             quote = "",
             header = FALSE)
colnames(dynamic_regulated_genes) <- "WBGeneID"</pre>
dynamic_regulated_genes %>% head
##
           WBGeneID
## 1 WBGene00004020
```

```
## 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
##
     <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

Make sure the column names are correct here and that the factor levels match the cluster description names

```
# temp_peaks <- mcols(readRDS("/Users/rtpw/Dropbox/01_GITHUBREPO/RWC23_elt2_regulated_genes/01_ChIPseq_
# write.csv(temp_peaks, file = "./01_input/201019_annotatedPeaks.csv")
library(GenomicRanges)
## Loading required package: stats4</pre>
```

```
## Loading required package: BiocGenerics
## Loading required package: parallel
##
## Attaching package: 'BiocGenerics'
## The following objects are masked from 'package:parallel':
##
       clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##
##
       clusterExport, clusterMap, parApply, parCapply, parLapply,
##
       parLapplyLB, parRapply, parSapply, parSapplyLB
##
  The following objects are masked from 'package:lubridate':
##
       intersect, setdiff, union
##
##
  The following objects are masked from 'package:dplyr':
##
##
       combine, intersect, setdiff, union
```

```
## The following objects are masked from 'package:stats':
##
       IQR, mad, sd, var, xtabs
##
## The following objects are masked from 'package:base':
##
##
       anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##
       dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
       grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##
       order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##
##
       rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
       union, unique, unsplit, which, which.max, which.min
##
## Loading required package: S4Vectors
##
## Attaching package: 'S4Vectors'
## The following objects are masked from 'package:lubridate':
##
##
       second, second<-
## The following objects are masked from 'package:dplyr':
##
##
       first, rename
## The following object is masked from 'package:tidyr':
##
##
       expand
## The following object is masked from 'package:base':
##
##
       expand.grid
## Loading required package: IRanges
##
## Attaching package: 'IRanges'
## The following object is masked from 'package:lubridate':
##
##
       %within%
## The following objects are masked from 'package:dplyr':
##
##
       collapse, desc, slice
## The following object is masked from 'package:purrr':
##
       reduce
## Loading required package: GenomeInfoDb
elt2_peaks <- as.data.frame(mcols(readRDS("/Users/rtpw/Dropbox/01_GITHUBREPO/RWC23_elt2_regulated_genes
elt2_peaks <- elt2_peaks %>% rename(k4labels = 'cluster.description', feature = "WBGeneID")
elt2_cluster_names <- c("Embryo_Specific",</pre>
                        "Larval",
                        "Increasing",
                        "L3_High",
```

```
"Not_Changing")
elt2_peaks$cluster.description <-</pre>
  factor(
    elt2_peaks$cluster.description,
    levels = c(
      "LE-specific",
      "Post-embryonic",
      "Increasing",
      "L3-high",
      "Not-changing or not IDR-passing"
    ),
    labels = elt2 cluster names
elt2_peaks %>% head
                     LE_1
                               LE_2
                                        L1_1
                                                 L1_2
                                                           L3_1
                                                                    L3_2 LE_IDR
## ELT2peak00001 1.932886 1.599462 4.253989 3.773996 4.884864 5.012244
                                                                               0
## ELT2peak00002 2.106915 1.941106 4.049631 4.456806 4.949960 5.939312
                                                                               0
## ELT2peak00003 1.222392 1.530515 2.613532 2.847997 2.447459 2.862497
                                                                               0
## ELT2peak00004 1.807355 1.415037 2.735522 3.280956 4.184424 4.491853
                                                                               0
## ELT2peak00005 2.222393 2.165059 2.244887 2.134878 4.019001 4.097713
                                                                               1
## ELT2peak00011 1.894818 2.103093 3.432960 2.847997 3.417085 3.534922
                 L1_IDR L3_IDR summit_agreement k4cluster k11cluster k4weights
                                        23.37500
                                                          4
                                                                    10
## ELT2peak00001
                       1
                              1
                                                                            0.967
## ELT2peak00002
                       1
                              1
                                         9.00000
                                                          4
                                                                    10
                                                                            0.996
## ELT2peak00003
                      0
                              1
                                       133.00000
                                                          2
                                                                     6
                                                                            0.535
                       0
                                                          4
                                                                    10
## ELT2peak00004
                              1
                                         4.00000
                                                                            0.898
                       1
                                                          3
                                                                     9
## ELT2peak00005
                              1
                                                                            0.989
                                        19.77778
                      0
                              1
                                       260.00000
                                                          4
                                                                     8
## ELT2peak00011
                                                                            0.895
##
                 k11weights LE_nonNormed L1_nonNormed L3_nonNormed LE_std L1_std
## ELT2peak00001
                      1.000
                                    1.766
                                                  4.014
                                                               4.949 -1.107 0.268
## ELT2peak00002
                      1.000
                                    2.024
                                                  4.253
                                                               5.445 -1.085 0.199
## ELT2peak00003
                      1.000
                                    1.376
                                                  2.731
                                                               2.655 -1.153 0.626
## ELT2peak00004
                       1.000
                                    1.611
                                                  3.008
                                                               4.338 -1.008 0.016
## ELT2peak00005
                       1.000
                                    2.194
                                                  2.190
                                                               4.058 -0.576 -0.579
## ELT2peak00011
                       1.000
                                    1.999
                                                  3.140
                                                               3.476 - 1.127
                                                                             0.347
                                  name cluster.description variance
                 L3_std
                                                                                peak
## ELT2peak00001 0.839 ELT2peak00001
                                                 Increasing 2.6756059 ELT2peak00001
## ELT2peak00002 0.885 ELT2peak00002
                                                 Increasing 3.0149205 ELT2peak00002
## ELT2peak00003
                  0.527 ELT2peak00003
                                                     Larval 0.5790878 ELT2peak00003
## ELT2peak00004
                  0.992 ELT2peak00004
                                                 Increasing 1.8594295 ELT2peak00004
## ELT2peak00005
                  1.155 ELT2peak00005
                                                    L3 High 1.1613434 ELT2peak00005
## ELT2peak00011
                  0.780 ELT2peak00011
                                                 Increasing 0.5995538 ELT2peak00011
##
                        WBGeneID start_position end_position feature_strand
## ELT2peak00001 WBGene00022277
                                                        10230
                                           4116
## ELT2peak00002 WBGene00022276
                                          11495
                                                        16837
## ELT2peak00003 WBGene00022276
                                          11495
                                                        16837
## ELT2peak00004 WBGene00022276
                                          11495
                                                        16837
## ELT2peak00005 WBGene00022276
                                          11495
                                                        16837
## ELT2peak00011 WBGene00021681
                                          96544
                                                       108223
##
                 insideFeature distancetoFeature shortestDistance
## ELT2peak00001
                    overlapEnd
                                             3219
                                                                102
```

```
## ELT2peak00002
                   overlapStart
                                              -2758
                                                                  263
## ELT2peak00003
                         inside
                                                 46
                                                                 1965
## ELT2peak00004
                         inside
                                               1253
                                                                 1179
## ELT2peak00005
                     overlapEnd
                                               2922
                                                                  135
## ELT2peak00011
                   overlapStart
                                              -6288
                                                                  156
                  fromOverlappingOrNearest
##
## ELT2peak00001
                                Overlapping
## ELT2peak00002
                                Overlapping
## ELT2peak00003
                                Overlapping
## ELT2peak00004
                                Overlapping
## ELT2peak00005
                                Overlapping
## ELT2peak00011
                                Overlapping
Make a set of genes with ELT-2 binding detected in the L1 stage.
elt2_detected_in_L1 <-</pre>
  elt2_peaks %>% select(WBGeneID, L1_IDR) %>% filter(L1_IDR == 1) %>% select(WBGeneID) %>% unique()
elt2_detected_in_L1 %>% head
##
           WBGeneID
## 1 WBGene00022277
## 2 WBGene00022276
## 4 WBGene00021026
## 5 WBGene00022037
## 6 WBGene00022042
## 7 WBGene00007009
elt2_detected_in_L1 %>% dim
## [1] 2319
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
## WBGene0000004
                                  0
                                         1
                                                     0
                                                              0
                                                                           0
## WBGene0000007
                                  0
                                         0
                                                     1
                                                              1
                                                                           0
## WBGene00000008
                                  0
                                         0
                                                     1
                                                              0
                                                                           0
## WBGene00000009
                                  0
                                                     0
                                                              0
                                                                           0
                                         1
## WBGene0000018
                                  0
                                         0
                                                     2
                                                              1
                                                                           1
## WBGene00000022
                                         0
                                                                           0
                                                              1
```

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 = "",</pre>
```

```
header = TRUE
 )
colnames(spencerLEgenes) <-</pre>
  str_c("spencer_LE_", colnames(spencerLEgenes))
spencer_LE_subset <-</pre>
  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
                                                                       13.86
## 2 WBGene00021252
                                   8.21
                                                             0
                                                                        7.30
                                   9.29
                                                            0
## 3 WBGene00019986
                                                                       10.67
## 4 WBGene00007904
                                   8.16
                                                            0
                                                                        6.89
## 5 WBGene00012018
                                  10.14
                                                            0
                                                                        6.25
## 6 WBGene00010540
                                   8.43
                                                                        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) <-</pre>
  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.

```
t(apply(unlist(dynamic_counts_matrix), 1, scale))
rownames(dynamic_counts_matrix_scaled) <-</pre>
  rownames(dynamic_counts_matrix)
colnames(dynamic_counts_matrix_scaled) <-</pre>
  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
## WBGene0000013
                   -1.0765042
                               0.04628523
                                            -1.0478603
                                                         -0.4296435
                                                                       -0.61401384
## WBGene0000016
                   -0.1629274
                               0.14035593
                                            -0.8318355
                                                         -0.2209018
                                                                        -0.52814604
## WBGene0000017
                                            -0.4453539
                    0.1344074 0.43209491
                                                          0.5202470
                                                                       -0.19720767
##
                  elt7D_sorted_2 elt7D_sorted_3 elt2D_sorted_1 elt2D_sorted_2
## WBGene0000007
                       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
## WBGene0000013
                     -0.58009755
                                     -0.38693983
                                                      -0.4767996
                                                                      0.3851813
## WBGene0000016
                      -0.50445577
                                     -0.16186256
                                                      -0.5681545
                                                                     -0.6137809
## WBGene0000017
                       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
                                                           -1.7004545
                      0.98161197
                                       1.7266509
## WBGene0000013
                      0.09286966
                                      -0.5163112
                                                            2.5457794
## WBGene0000016
                     -0.75209134
                                      -1.0136068
                                                            1.7015008
## WBGene0000017
                     -1.16996644
                                      -1.7376299
                                                            1.4066491
##
                  elt2Delt7D_sorted_2 elt2Delt7D_sorted_3
## WBGene0000007
                            -0.8564679
                                                 -1.1220323
## WBGene00000008
                            -0.5550156
                                                 -0.8273297
## WBGene00000009
                            -0.8668929
                                                 -1.4597714
## WBGene0000013
                             1.4999051
                                                 0.5581492
## WBGene0000016
                             2.1353949
                                                  1.3805110
## WBGene0000017
                                                  0.9767996
                             1.6701858
dynamic_counts_matrix_scaled_ascend <-</pre>
  dynamic_counts_matrix_scaled[order(rownames(dynamic_counts_matrix_scaled)),]
```

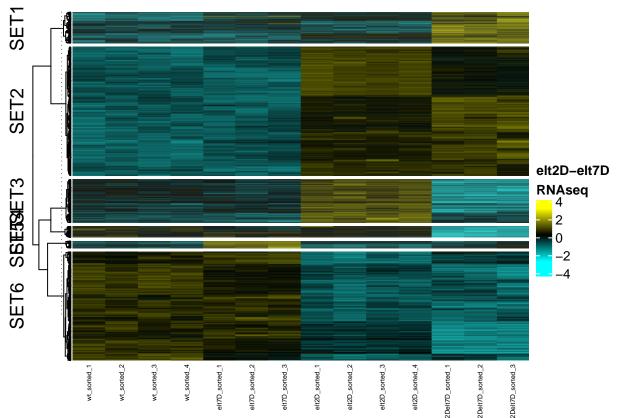
Must use arrange to sort genes in descending order to ensure row order is preserved

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
)
```

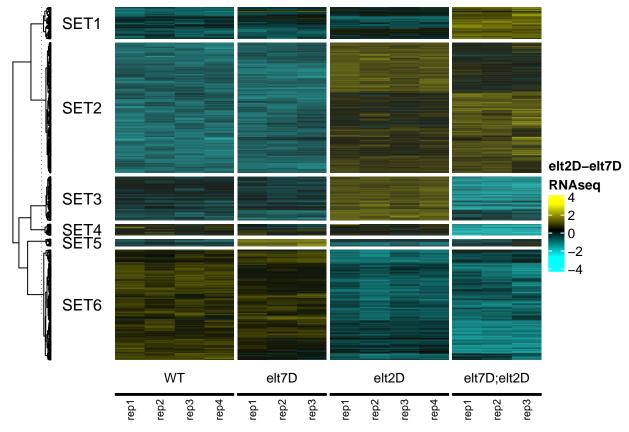


Add expression set and column labels.

```
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
                                                                 elt7Delt2D
                   elt2D
                              elt2D
                                          elt2D
                                                     elt2D
## [13] elt7Delt2D elt7Delt2D
## Levels: WT elt7D elt2D elt7Delt2D
column_labels <-
  structure(
    c(
```

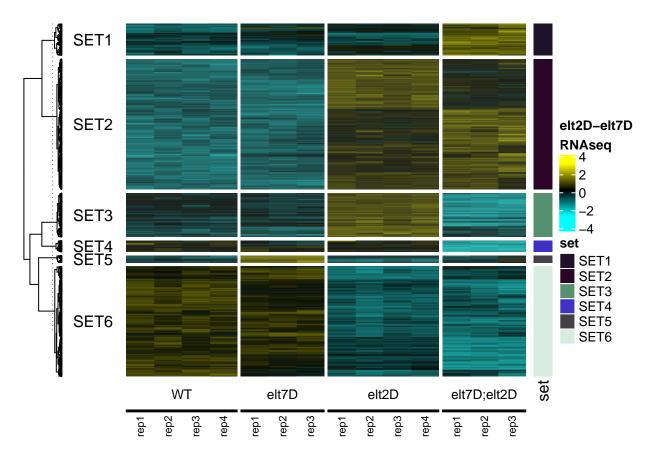
```
"rep1",
      "rep2",
      "rep3",
      "rep4",
      "rep1",
      "rep2",
      "rep3",
      "rep1",
      "rep2",
      "rep3",
      "rep4",
      "rep1",
      "rep2",
      "rep3"
    ),
    names = colnames(dynamic_counts_matrix_scaled_ascend)
  )
column_labels
##
                                wt_sorted_2
                                                    wt_sorted_3
                                                                         wt_sorted_4
           wt_sorted_1
##
                "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(</pre>
  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

1 WBGene0000007

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.

```
## 2 WBGene00000008
                                   bound
## 3 WBGene00000009
                               not.bound
## 4 WBGene0000013
                               not.bound
## 5 WBGene00000016
                               not.bound
## 6 WBGene0000017
                               not.bound
Incorporate this into a heatmap annotation
Ha_L1chip <-</pre>
  Ha + rowAnnotation(L1bound = elt2_L1_anno$elt2_detected_in_L1,
                     col = list(L1bound = c(
                        "bound" = "green", "not.bound" = "black"
                     )))
Ha_L1chip
       SET1
       SET2
                                                                               elt2D-elt7D
                                                                               RNAseq
                                                                                  2
       SET3
                                                                                  0
                                                                                  -2
                                                                               L1bound
                                                                                 bound
                                                                                 not.bound
       SET6
                      WT
                                                   elt2D
                                                               elt7D;elt2D
                                    elt7D
if (plot == TRUE){
myPDFplot(Ha_L1chip, "01a_DE_Heatmap_elt2elt7DERNAseq_L1elt2bound", 4, 4.5, plotdir)
}
## pdf
##
Add Spencer intestine data
```

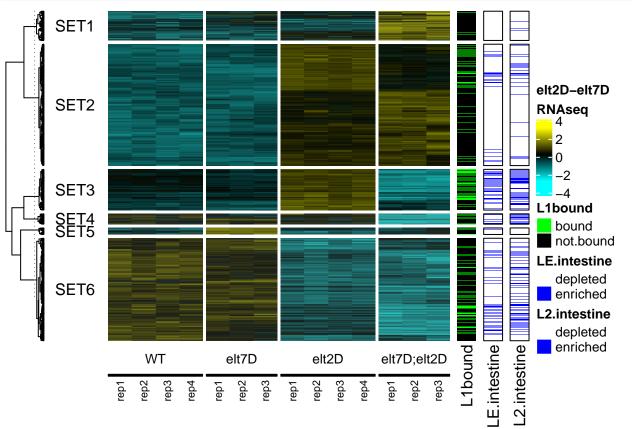
test = rownames(dynamic_counts_matrix_scaled_ascend) %in% spencer_LE_subset\$spencer_LE_ID,

spencer_rna_anno <- data.frame(</pre>

spencerLE = ifelse(

yes = "enriched",
no = "depleted"

```
),
  spencerL2 = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_ascend) %in% spencer_L2_subset$spencer_L2_ID,
    yes = "enriched",
    no = "depleted"
  )
)
Ha_L1chip_spencer <- Ha_L1chip +</pre>
  rowAnnotation(
    LE.intestine = spencer_rna_anno$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,
}
## pdf
## 2
```

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 assessment.

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 WBGene00000009 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</pre>
```

```
##
##
           bound not.bound
##
     SET1
              26
                         265
##
     SET2
             165
                        1043
             168
                         237
##
     SET3
##
              38
                          65
     SET4
##
     SET5
              17
                          48
     SET6
                         772
##
             248
```

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

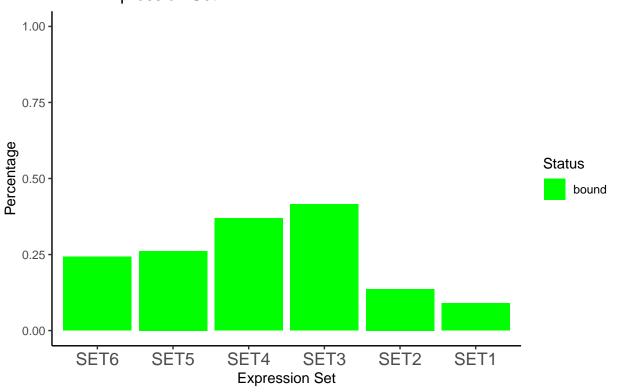
```
clust_L1bound_prop <- prop.table(clust_L1bound_counts, 1)
clust_L1bound_prop</pre>
```

```
## bound not.bound
## SET1 0.08934708 0.91065292
## SET2 0.13658940 0.86341060
## SET3 0.41481481 0.58518519
## SET4 0.36893204 0.63106796
## SET5 0.26153846 0.73846154
## SET6 0.24313725 0.75686275
```

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

```
clust_L1bound_prop_ggplot <- as.data.frame(clust_L1bound_prop)</pre>
colnames(clust_L1bound_prop_ggplot) <- c("SET", "Status", "Freq")</pre>
clust_L1bound_prop_ggplot$Status <-</pre>
  factor(clust_L1bound_prop_ggplot$Status,
         levels = c("not.bound", "bound"))
clust_L1bound_prop_ggplot$SET <-</pre>
  factor(
    clust_L1bound_prop_ggplot$SET,
    levels = c("SET6", "SET5", "SET4", "SET3", "SET2", "SET1")
 )
clust_L1bound_colors <- c("bound" = "green", "not.bound" = "black")</pre>
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)
11bound_percents
```

Percentage of L1 Stage ELT–2 Binding Per Expression Set

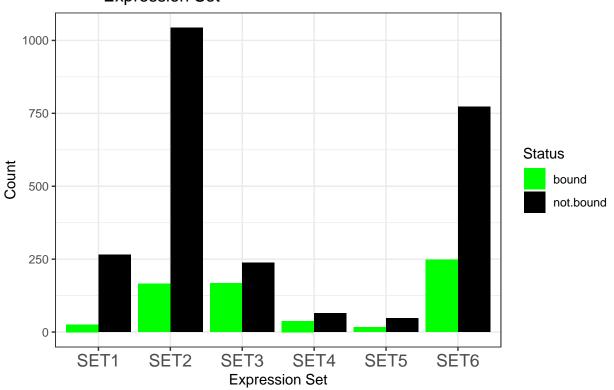


This plot shows that all of the differential expression sets have less than 50% of genes bound by ELT-2.

Rather than viewing percentages of genes bound, what is the number of "bound" vs "not.bound" per cluster?

bound_per_cluster

Number of L1 Stage ELT–2 Binding Site Per Expression Set



```
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] 0.2141009

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

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

```
## Set pval conf.lower conf.upper bool
```

```
## 1 SET1 1.745474e-08 0.0591957 0.1281721 TRUE

## 2 SET2 6.470997e-12 0.1177126 0.1572542 TRUE

## 3 SET3 1.289155e-19 0.3663731 0.4645105 TRUE

## 4 SET4 4.072977e-04 0.2759462 0.4696653 TRUE

## 5 SET5 3.640163e-01 0.1603288 0.3853937 FALSE

## 6 SET6 2.676330e-02 0.2170935 0.2706723 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 1.027355e-08
                               0 0.1218068
## 2 SET2 3.135413e-12
                               0 0.1539130 TRUE
## 3 SET3 1.000000e+00
                               0 0.4566871 FALSE
## 4 SET4 9.998953e-01
                               0 0.4541133 FALSE
## 5 SET5 8.600190e-01
                               0 0.3660888 FALSE
## 6 SET6 9.883920e-01
                               0 0.2662563 FALSE
```

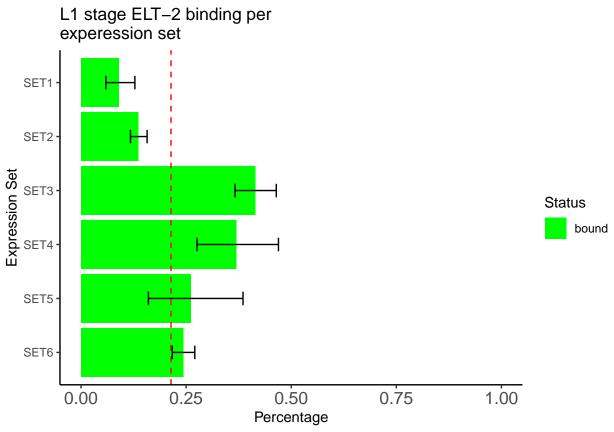
This says that set 1 and 2 have less ELT-2 binding compared to the entire dataset.

Now try greater.

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

This says that SET3, SET4 and SET6 have a higher percentage of genes bound compared 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.



```
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)</pre>
```

[1] 4.001798e-97

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 4.652e-01
## SET3 1.005e-21 7.631e-29
## SET4 8.177e-09 5.099e-07 1.000e+00
## SET5 6.956e-03 1.445e-01 3.013e-01 1.0000
## SET6 2.360e-08 1.905e-09 4.443e-09 0.1293
##
## 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
                    TRUE FALSE FALSE
         TRUE
               TRUE
```

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.

```
##
           WBGeneID Embryo_Specific Larval Increasing L3_High Not_Changing
## 1 WBGene0000007
                                    0
                                                        1
                                                                 1
                                                                               0
                                    0
                                                                               0
## 2 WBGene00000008
                                            0
                                                        1
                                                                 0
## 3 WBGene00000009
                                    0
                                                                               0
                                            1
                                                        0
                                                                 0
## 4 WBGene0000013
                                    0
                                            0
                                                        0
                                                                 0
                                                                               0
## 5 WBGene00000016
                                    0
                                            0
                                                        0
                                                                 0
                                                                               0
## 6 WBGene0000017
                                                                               0
```

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

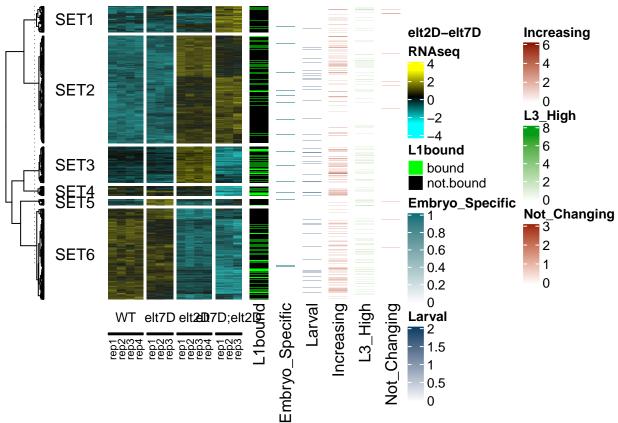
```
unique(rownames(dynamic_counts_matrix_scaled_ascend) == chip_annotation$WBGeneID)
```

```
## [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) +</pre>
```

```
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
```



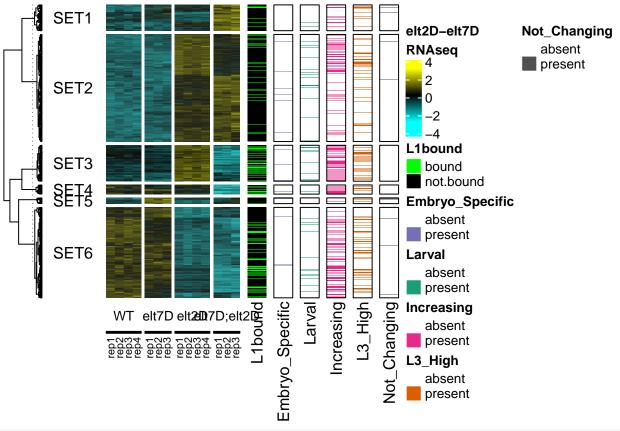
Have the colors match plot from David.

Convert ChIP binding clusters to a present/absence list.

```
chip_annotation_present_absent <-
make_cluster_binary_annotation(chip_annotation)</pre>
```

Plot the heatmap with presence/absence.

```
Ha_L1chip_clusterchip <-
   Ha_L1chip + binding_cluster_row_annotation(chip_annotation_present_absent)</pre>
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
)
}
```

pdf ## 2

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"
    )),</pre>
```

```
border = TRUE
  )
Ha_L1chip_clusterchip_spencerRNA
        SET1
                                                                  elt2D-elt7D
                                                                                      Not_Changing
                                                                                         absent
                                                                  RNAseq
                                                                     4
                                                                                       present
                                                                     2
                                                                                      LE.intestine
        SET2
                                                                     0
                                                                                         depleted
                                                                      -2
                                                                                      enriched
                                                                                      L2.intestine
                                                                  L1bound
                                                                                         depleted
        SET3
                                                                     bound
                                                                                        enriched
                                                                   not.bound
        SET4
                                                                  Embryo_Specific
                                                                     absent
                                                                     present
        SET6
                                                                  Larval
                                                                     absent
                                                                     present
                W Telt7 6 t 1200; elt 200 o q 1
                                             Increasing [
                                         Larval
                                                 L3_High[
                                                      Not_Changing[
                                                          LE.intestine
                                    Embryo_Specific
                                                              L2.intestine
                                                                  Increasing
                                                                     absent
                                                                    present
                                                                  L3_High
                                                                     absent
                                                                     present
if (plot == TRUE){
  myPDFplot(
    plot = Ha_L1chip_clusterchip_spencerRNA,
    name = "05b_DE_Heatmap_L1elt2bound_elt2bindclusters_spencerRNA_anno",
    height = 6.5,
    width = 8,
    plotdir = plotdir
  )
  }
## pdf
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 present
                                                                          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
                                                     absent absent
## 6 WBGene00000017 SET1
                                  absent absent
                                                                          absent
```

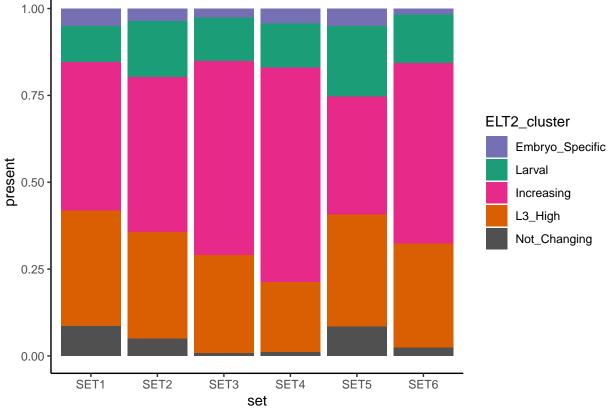
What is the percentage of genes with annotated ELT2 binding clusters per expression dataset?

Make a dataframe that addresses the question:

```
##
       set absent present
                              ELT2 cluster
                                                percent
## 1 SET1
              285
                        6 Embryo_Specific 0.020618557
## 2 SET2
             1187
                        21 Embryo_Specific 0.017384106
## 3
     SET3
              395
                       10 Embryo_Specific 0.024691358
## 4
     SET4
               99
                         4 Embryo_Specific 0.038834951
## 5
     SET5
               62
                         3 Embryo_Specific 0.046153846
## 6
     SET6
             1008
                        12 Embryo_Specific 0.011764706
## 7
      SET1
              279
                        12
                                    Larval 0.041237113
## 8
     SET2
             1113
                       95
                                    Larval 0.078642384
## 9 SET3
              355
                       50
                                    Larval 0.123456790
## 10 SET4
                       12
               91
                                    Larval 0.116504854
## 11 SET5
               53
                        12
                                    Larval 0.184615385
## 12 SET6
              926
                       94
                                    Larval 0.092156863
## 13 SET1
              241
                       50
                                Increasing 0.171821306
## 14 SET2
              946
                      262
                                Increasing 0.216887417
## 15 SET3
              184
                       221
                                Increasing 0.545679012
## 16 SET4
               45
                                Increasing 0.563106796
                       58
## 17 SET5
                                Increasing 0.307692308
               45
                       20
## 18 SET6
              669
                       351
                                Increasing 0.344117647
## 19 SET1
              252
                       39
                                   L3_High 0.134020619
```

```
L3_High 0.149834437
## 20 SET2
             1027
                       181
## 21 SET3
              293
                       112
                                   L3_High 0.276543210
## 22 SET4
               84
                       19
                                   L3_High 0.184466019
               46
                                   L3_High 0.292307692
## 23 SET5
                       19
## 24 SET6
              817
                       203
                                   L3_High 0.199019608
## 25 SET1
              281
                        10
                              Not_Changing 0.034364261
## 26 SET2
                        29
                              Not Changing 0.024006623
             1179
## 27 SET3
                              Not_Changing 0.007407407
              402
                        3
## 28 SET4
              102
                        1
                              Not_Changing 0.009708738
## 29 SET5
               60
                              Not_Changing 0.076923077
                         5
## 30 SET6
             1004
                        16
                              Not_Changing 0.015686275
```

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

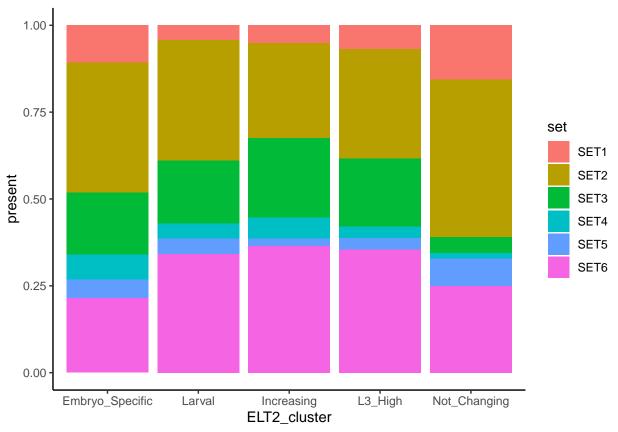


```
# ggsave("./03_plots/06_Cluster_percent_present_per_Set_200615.pdf")

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?

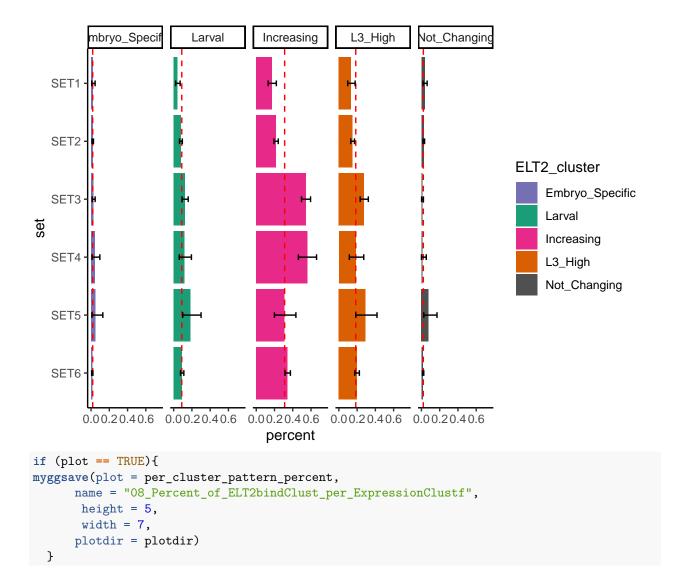


Make a series of horizontal barplots with percentage of ELT-2 binding cluster per expression cluster.

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

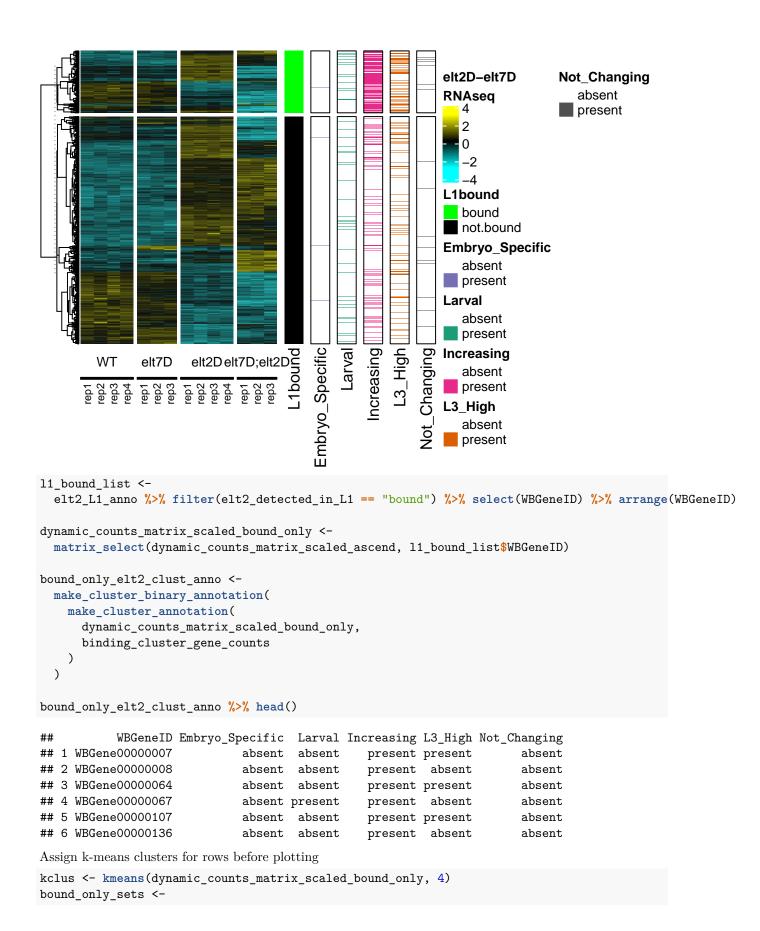
```
percent_bound_per_ELT2_cluster <-</pre>
  expressionSet_per_BindingCluster %>% group_by(ELT2_cluster) %>% summarise(percent = sum(present) /
                                                                               nrow(dynamic counts matri:
Next calculate the the 95% Confidence Interval with 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
                             0.0412
## 3 SET1 Increasing
                             0.172
## 4 SET1 L3_High
                             0.134
## 5 SET1 Not_Changing
                             0.0344
## 6 SET2 Embryo_Specific 0.0174
## 7 SET2 Larval
                             0.0786
## 8 SET2 Increasing
                             0.217
## 9 SET2 L3_High
                             0.150
## 10 SET2 Not_Changing
                             0.0240
## # ... 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]
##
          absent present ELT2_cluster percent background_perc~ pval conf.upper
     set
##
     <fct> <int>
                  <int> <fct>
                                         <dbl>
                                                          <dbl> <dbl>
                                                                           <dbl>
## 1 SET1
             285
                       6 Embryo_Spec~ 0.0206
                                                         0.0181 0.658
                                                                          0.0443
## 2 SET2
            1187
                       21 Embryo_Spec~ 0.0174
                                                         0.0181 1
                                                                          0.0265
            395
## 3 SET3
                     10 Embryo_Spec~ 0.0247
                                                         0.0181 0.346
                                                                          0.0449
## 4 SET4
             99
                       4 Embryo Spec~ 0.0388
                                                         0.0181 0.118
                                                                          0.0965
## 5 SET5
              62
                        3 Embryo_Spec~ 0.0462
                                                         0.0181 0.114
                                                                          0.129
## 6 SET6
             1008
                       12 Embryo_Spec~ 0.0118
                                                         0.0181 0.157
                                                                          0.0205
## # ... with 1 more variable: conf.lower <dbl>
per_cluster_pattern_percent <- ggplot(expression_binding_stats,</pre>
       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
```



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)
```



```
data.frame(
    WBGeneID = rownames(dynamic_counts_matrix_scaled_bound_only),
    set = paste("SET", kclus$cluster, sep = "")
  )
head(bound_only_sets)
##
           WBGeneID set
## 1 WBGene00000007 SET2
## 2 WBGene00000008 SET2
## 3 WBGene00000064 SET3
## 4 WBGene00000067 SET1
## 5 WBGene00000107 SET2
## 6 WBGene00000136 SET2
Draw heatmap and check that set assignment is correct.
Ha_bound_only <-</pre>
  RNA_heatmap2(mat = dynamic_counts_matrix_scaled_bound_only,
              column_split = RNA_column_order,
              row_split = bound_only_sets$set,
              row_title = c("SET3", "SET4", "SET1", "SET2"))
Ha_bound_only
SET3
SET4
                                                                               elt2D-elt7D
                                                                               RNAseq
SET1
                                                                                  2
                                                                                  0
                                                                                  -2
                 WT
                                  elt7D
                                                   elt2D
                                                                  elt7D;elt2D
if (plot == TRUE){
  myPDFplot(
    plot = Ha_bound_only,
    name = "09a_DE_Heatmap_L1elt2boundOnly",
```

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

## pdf
## 2
```

TODO: ROTATE CLUSTERS TO MAKE SENSE

```
Ha_bound_only +
  rowAnnotation(sets = bound_only_sets$set)
SET3
SET4
                                                                                elt2D-elt7D
                                                                                RNAseq
                                                                                  2
                                                                                  0
SET1
                                                                                   -2
                                                                               sets
                                                                                  SET1
                                                                                  SET2
                                                                                  SET3
SET2
                                                                                  SET4
                 WT
                                 elt7D
                                                 elt2D
                                                               elt7D;elt2D
               rep2
                         rep4
                                  rep2
           ep1
                              ep1
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 SET2
                              absent
                                                 present present
## 2 WBGene00000008
                              absent
                                       absent
                                                 present absent
                                                                        absent SET2
## 3 WBGene0000064
                              absent absent
                                                 present present
                                                                        absent SET3
```

present absent

absent SET1

absent present

4 WBGene00000067

```
## 5 WBGene00000107
                                absent absent
                                                                              absent SET2
                                                     present present
## 6 WBGene00000136
                                absent absent
                                                                              absent SET2
                                                     present absent
Ha_bound_only_chipClust <-</pre>
  Ha_bound_only + binding_cluster_row_annotation(bound_only_elt2_clust_anno)
Ha_bound_only_chipClust
SET3
                                                                               elt2D-elt7D
                                                                               RNAseq
SET4
                                                                                  2
                                                                                  0
                                                                                   -2
                                                                               Embryo_Specific
SET1
                                                                                  absent
                                                                                present
                                                                               Larval
                                                                                  absent
                                                                                 present
SET2
                                                                               Increasing
                                                                                  absent
                                                                                  present
                                                               Larval
                                                                       L3_High
                                                                            D absen preser Not_Cha
                                                                   Increasing
                                                           Embryo_Specific
               WT
                          elt7D
                                      elt2D
                                               elt7D;elt2D
                                                                                  absent
                                                      rep3
                                                                                  present
                                                rep1
                                                                               Not_Changing
                                                                                  absent
                                                                                  present
if (plot == TRUE){
  myPDFplot(
    plot = Ha_bound_only_chipClust,
    name = "09b_DE_Heatmap_L1elt2boundOnly_elt2bindclusters_anno",
    height = 6.5,
    width = 6,
    plotdir = plotdir
## pdf
##
```

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"
),</pre>
```

```
spencerL2 = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_bound_only) %in% spencer_L2_subset$spencer_L2_ID,
    yes = "enriched",
    no = "depleted"
  )
)
Ha_bound_only_chipClust_spencer <- Ha_bound_only_chipClust +</pre>
  rowAnnotation(
    LE.intestine = bound_only_spencer_rna_anno$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
SET3
                                                              elt2D-elt7D
                                                                               LE.intestine
                                                                                  depleted
                                                              RNAseq
                                                                4
                                                                                enriched
SET4
                                                                2
                                                                               L2.intestine
                                                                0
                                                                                  depleted
                                                                                enriched
                                                              Embryo_Specific
SET1
                                                                absent
                                                              present
                                                              Larval
                                                                 absent
                                                               present
SET2
                                                              Increasing
                                                                 absent
                                                               present
               Embryo_Spec
                                          Increasing
                                              L3_High
                                                      LE.intestine
                                                  Not_Changing
                                                          L2.intestine
                                                             L3_High
                                                                 absent
         present
                                                              Not_Changing
                                                                 absent
                                                                present
if (plot == TRUE){
  myPDFplot(
```

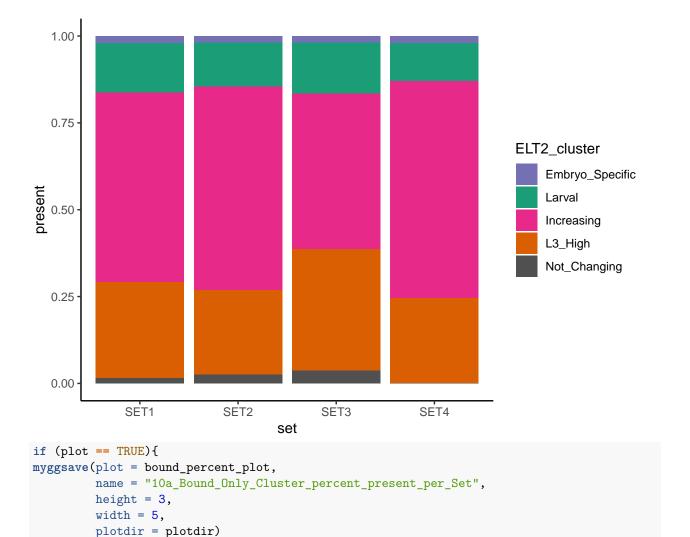
```
plot = Ha_bound_only_chipClust_spencer,
   name = "09c_DE_Heatmap_L1elt2boundOnly_elt2bindclusters_spencerRNA",
   height = 6.5,
   width = 6,
   plotdir = plotdir
 }
## pdf
##
What is the percentage of genes with annotated ELT2 binding clusters per ex-
pression dataset?
bound only exprclust bindclust <-
  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 SET2
                                 absent absent
                                                    present present
                                                                          absent
## 2 WBGene00000008 SET2
                                  absent absent
                                                    present absent
                                                                          absent
## 3 WBGene00000064 SET3
                                 absent absent
                                                    present present
                                                                          absent
## 4 WBGene00000067 SET1
                                                                          absent
                                 absent present
                                                    present absent
## 5 WBGene00000107 SET2
                                  absent absent
                                                    present present
                                                                          absent
## 6 WBGene00000136 SET2
                                  absent absent
                                                    present absent
                                                                          absent
Make a dataframe that addresses the question:
bound_only_expressionSet_per_BindingCluster <- data.frame()</pre>
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

```
## 4 SET4
              143
                        4 Embryo_Specific 0.02721088
## 5 SET1
              110
                       29
                                    Larval 0.20863309
## 6 SET2
              196
                       39
                                    Larval 0.16595745
     SET3
              109
                       32
                                    Larval 0.22695035
## 7
## 8 SET4
              125
                       22
                                    Larval 0.14965986
## 9 SET1
               29
                      110
                                Increasing 0.79136691
## 10 SET2
               53
                      182
                                Increasing 0.77446809
                                Increasing 0.68794326
## 11 SET3
               44
                       97
## 12 SET4
               22
                      125
                                Increasing 0.85034014
## 13 SET1
                       56
                                   L3_High 0.40287770
               83
## 14 SET2
              160
                       75
                                   L3_High 0.31914894
## 15 SET3
                       76
                                   L3_High 0.53900709
               65
## 16 SET4
                                   L3_High 0.33333333
               98
                       49
## 17 SET1
              136
                        3
                             Not_Changing 0.02158273
## 18 SET2
              227
                        8
                             Not_Changing 0.03404255
## 19 SET3
              133
                         8
                             Not_Changing 0.05673759
## 20 SET4
              147
                         0
                             Not_Changing 0.00000000
```

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

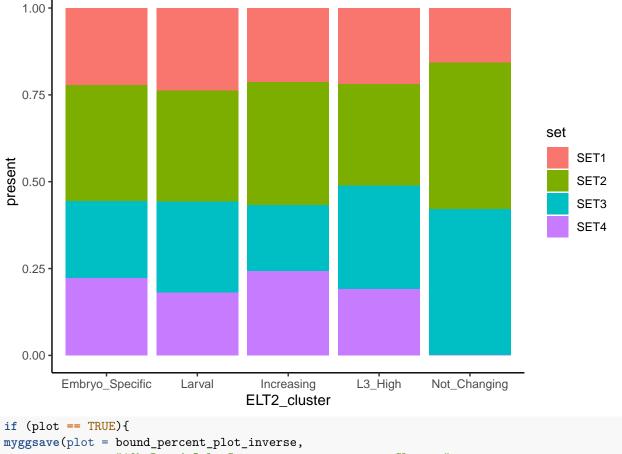
```
bound_percent_plot <- ggplot(
  bound_only_expressionSet_per_BindingCluster,
  aes(x = set,
      y = present,
      fill = ELT2_cluster)
) +
  geom_bar(stat = "identity", position = "fill") +
  theme_classic() +
  scale_fill_manual(values = as.vector(cluster_colors$val))</pre>
```



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</pre>
```



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.

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

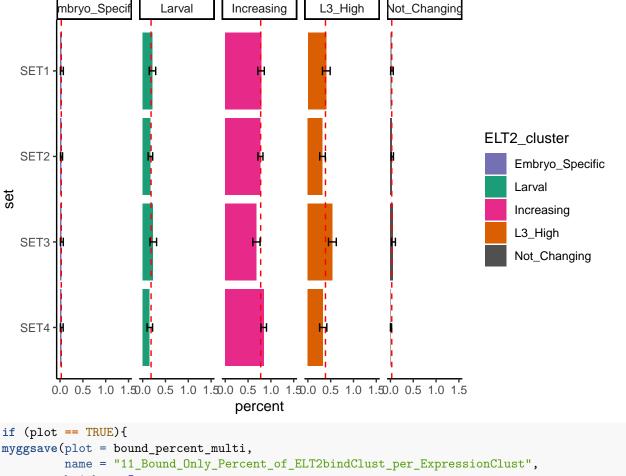
```
## # A tibble: 20 x 3
               set [4]
## # Groups:
           ELT2_cluster
##
      set
                            percent
##
      <chr> <fct>
                              <dbl>
##
   1 SET1 Embryo_Specific 0.0288
                             0.209
   2 SET1 Larval
                             0.791
   3 SET1
           Increasing
   4 SET1 L3_High
                             0.403
```

```
## 6 SET2 Embryo_Specific 0.0255
                             0.166
## 7 SET2 Larval
## 8 SET2 Increasing
                             0.774
## 9 SET2 L3_High
                             0.319
## 10 SET2 Not_Changing
                             0.0340
## 11 SET3
           Embryo_Specific 0.0284
## 12 SET3 Larval
                             0.227
## 13 SET3 Increasing
                             0.688
## 14 SET3 L3_High
                             0.539
## 15 SET3 Not_Changing
                             0.0567
## 16 SET4 Embryo_Specific
                             0.0272
## 17 SET4 Larval
                             0.150
## 18 SET4
           Increasing
                             0.850
## 19 SET4 L3_High
                             0.333
## 20 SET4
           Not_Changing
                             0
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) + s
# Use binom.test to calculate pualue 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()
```

5 SET1 Not_Changing

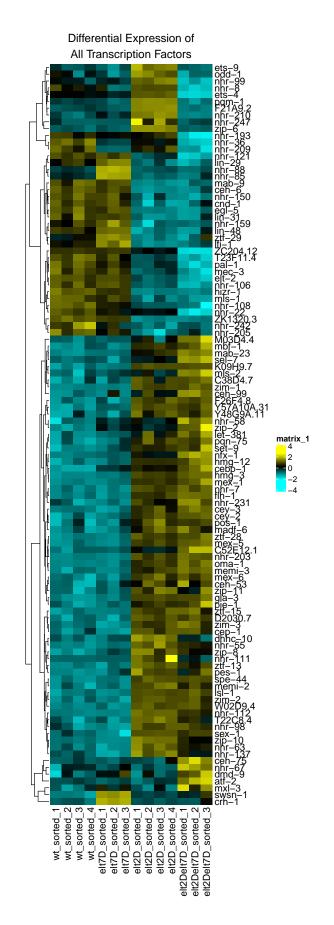
0.0216

```
## # A tibble: 6 x 9
## # Groups: ELT2_cluster, set [6]
         absent present ELT2_cluster percent background_perc~ pval conf.upper
                                                        <dbl> <dbl>
     <fct> <int> <int> <fct>
                                                                          <dbl>
##
                                        <dbl>
## 1 SET1
             135
                       4 Embryo_Spec~ 0.0288
                                                        0.0272 0.793
                                                                         0.0720
## 2 SET2
             229
                       6 Embryo Spec~ 0.0255
                                                        0.0272 1
                                                                         0.0547
## 3 SET3
            137
                       4 Embryo_Spec~ 0.0284
                                                        0.0272 0.795
                                                                         0.0710
## 4 SET4
                       4 Embryo_Spec~ 0.0272
                                                        0.0272 1
                                                                         0.0682
            143
## 5 SET1
             110
                      29 Larval
                                       0.209
                                                        0.184 0.445
                                                                         0.286
## 6 SET2
             196
                      39 Larval
                                       0.166
                                                        0.184 0.502
                                                                         0.220
## # ... with 1 more variable: conf.lower <dbl>
bound_percent_multi <- ggplot(bound_only_expression_binding_stats,</pre>
       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_expression_binding_stats %>% ungroup() %>% select(ELT2_cluster, background_percen
   color = "red",
   linetype = "dashed",
   aes(yintercept = background_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
```



Make a TF subset heatmap

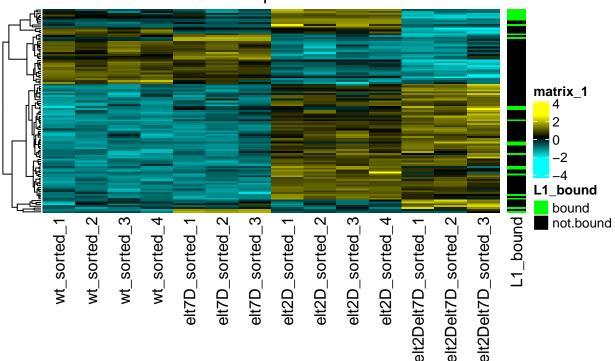
```
clustering_method_rows = "complete",
  show_row_names = TRUE,
  show_column_names = TRUE,
  column_title = "Differential Expression of\nAll Transcription Factors"
)
tf_heatmap
```



```
if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap,
    name = "12_Differential_Expression_of_All_TFs",
    height = 20,
    width = 4,
    plotdir = plotdir
  )
  }
## pdf
##
Add row annotation to indicate ELT-2 binding in L1 stage
elt2_detected_in_L1 %>% filter(WBGeneID %in% rownames(dynamic_counts_matrix_scaled_TFs))
## 1 WBGene00004096
## 2 WBGene00019327
## 3 WBGene00003711
     WBGene00000793
## 5 WBGene00021082
## 6 WBGene00003607
## 7
      WBGene00019743
      WBGene00003689
## 8
## 9 WBGene00003645
## 10 WBGene00003648
## 11 WBGene00012101
## 12 WBGene00014193
## 13 WBGene00016997
## 14 WBGene00018704
## 15 WBGene00016865
## 16 WBGene00019344
## 17 WBGene00017687
## 18 WBGene00003727
## 19 WBGene00003511
## 20 WBGene00003678
## 21 WBGene00016888
## 22 WBGene00003845
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
```

Differential Expression of All Transcription Factors



```
# 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
)
}
```

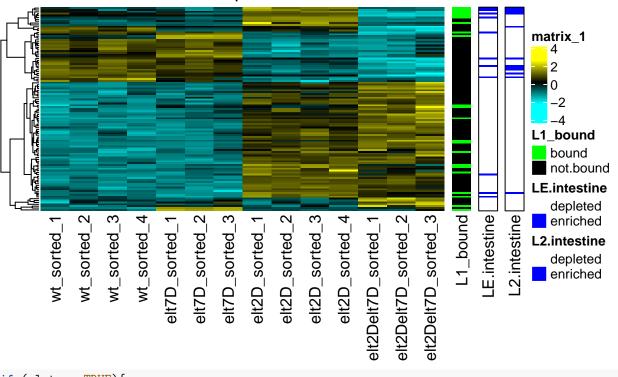
pdf ## 2

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"
    ),</pre>
```

```
spencerL2 = ifelse(
    test = rownames(dynamic_counts_matrix_scaled_TFs) %in% spencer_L2_subset$spencer_L2_ID,
    yes = "enriched",
    no = "depleted"
  )
)
tf_heatmap_L1bound_spencerRNA <- tf_heatmap_L1bound + rowAnnotation(</pre>
    LE.intestine = tf_spencer_rna_anno$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
```

Differential Expression of All Transcription Factors

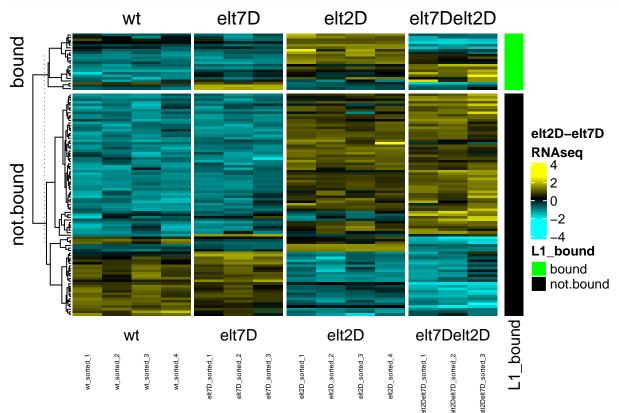


```
if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap_L1bound_spencerRNA,
    name = "13b_Differential_Expression_of_All_TFs_L1elt2bound_anno",
```

```
height = 5,
width = 5.5,
plotdir = plotdir
)
}
```

pdf ## 2

Split heatmap based on L1 binding

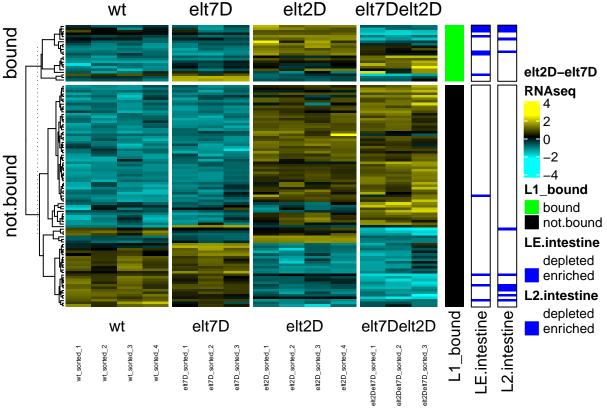


```
if (plot == TRUE){
  myPDFplot(
    plot = tf_heatmap_L1bound_split,
    name = "14a_Differential_Expression_of_All_TFs_L1elt2bound_split",
    height = 5,
    width = 5.5,
    plotdir = plotdir
)
}
```

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

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

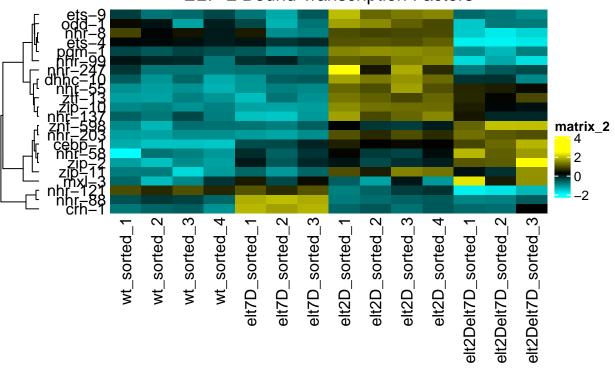
```
tf_heatmap_L1bound_split_spencerRNA <- tf_heatmap_L1bound_split +</pre>
  rowAnnotation(
    LE.intestine = tf_spencer_rna_anno$spencerLE,
    col = list(LE.intestine = c(
      "enriched" = "blue", "depleted" = "white"
    )),
    border = TRUE
  ) +
  rowAnnotation(
    L2.intestine = tf_spencer_rna_anno$spencerL2,
    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,
```

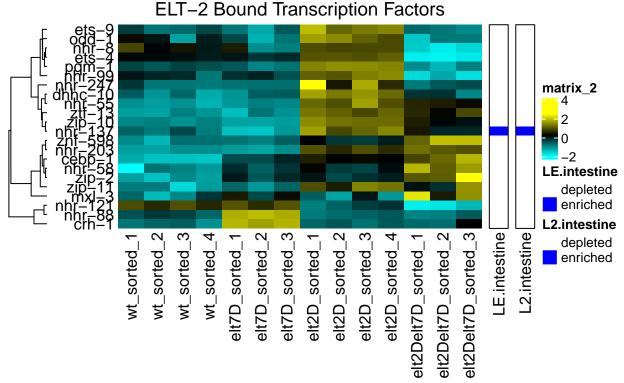
```
plotdir = plotdir
  )
## pdf
##
Zoom in on only bound TFs
dynamic_counts_matrix_scaled_TFs_bound <-</pre>
  matrix_select(dynamic_counts_matrix_scaled_TFs,
                 elt2_detected_in_L1$WBGeneID)
dynamic_counts_matrix_scaled_TFs_bound_names <-</pre>
  id2name(dynamic_counts_matrix_scaled_TFs_bound)
HAboundTF <- Heatmap(</pre>
  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
```

Differential Expression of ELT–2 Bound Transcription Factors



```
if (plot == TRUE){
  myPDFplot(
    plot = HAboundTF,
    name = "15a_Differential_Expression_Bound_TFs_only",
    height = 5,
    width = 5.5,
    plotdir = plotdir
  )
  }
## pdf
##
tf_bound_spencer_rna_anno <- data.frame(</pre>
  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(</pre>
    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
```

Differential Expression of



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

pdf ## 2

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

Scratch

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16067 / R16067C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16068 / R16068C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16069 / R16069C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16070 / R16070C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16071 / R16071C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16072 / R16072C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16073 / R16073C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16074 / R16074C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16075 / R16075C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16076 / R16076C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16077 / R16077C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16078 / R16078C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16079 / R16079C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16080 / R16080C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16081 / R16081C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16082 / R16082C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16083 / R16083C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16084 / R16084C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16085 / R16085C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16086 / R16086C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16087 / R16087C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16088 / R16088C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16089 / R16089C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16090 / R16090C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16091 / R16091C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16092 / R16092C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16093 / R16093C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16094 / R16094C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16095 / R16095C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16096 / R16096C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16097 / R16097C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16098 / R16098C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16099 / R16099C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16100 / R16100C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16101 / R16101C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16102 / R16102C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16103 / R16103C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16104 / R16104C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16105 / R16105C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16106 / R16106C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16107 / R16107C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16108 / R16108C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16109 / R16109C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16110 / R16110C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16111 / R16111C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16112 / R16112C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16113 / R16113C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16114 / R16114C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16115 / R16115C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16116 / R16116C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16117 / R16117C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16118 / R16118C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16119 / R16119C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16120 / R16120C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16121 / R16121C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16122 / R16122C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16123 / R16123C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16124 / R16124C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16125 / R16125C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16126 / R16126C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16127 / R16127C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16128 / R16128C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16129 / R16129C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16130 / R16130C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16131 / R16131C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16132 / R16132C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16133 / R16133C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16134 / R16134C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16135 / R16135C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16136 / R16136C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16137 / R16137C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16138 / R16138C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16139 / R16139C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16140 / R16140C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16141 / R16141C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16142 / R16142C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16143 / R16143C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16144 / R16144C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16145 / R16145C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16146 / R16146C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16147 / R16147C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16148 / R16148C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16149 / R16149C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16150 / R16150C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16151 / R16151C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16152 / R16152C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16153 / R16153C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16154 / R16154C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16155 / R16155C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16156 / R16156C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16157 / R16157C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16158 / R16158C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16159 / R16159C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16160 / R16160C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16161 / R16161C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16162 / R16162C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16163 / R16163C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16164 / R16164C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16165 / R16165C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16166 / R16166C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16167 / R16167C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16168 / R16168C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16169 / R16169C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16170 / R16170C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16171 / R16171C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16172 / R16172C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16173 / R16173C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16174 / R16174C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16175 / R16175C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16176 / R16176C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16177 / R16177C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16178 / R16178C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16179 / R16179C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16180 / R16180C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16181 / R16181C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16182 / R16182C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16183 / R16183C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16184 / R16184C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16185 / R16185C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16186 / R16186C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16187 / R16187C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16188 / R16188C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16189 / R16189C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16190 / R16190C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16191 / R16191C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16192 / R16192C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16193 / R16193C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16194 / R16194C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16195 / R16195C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16196 / R16196C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16197 / R16197C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16198 / R16198C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16199 / R16199C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16200 / R16200C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16201 / R16201C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16202 / R16202C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16203 / R16203C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16204 / R16204C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16205 / R16205C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16206 / R16206C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16207 / R16207C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16208 / R16208C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16209 / R16209C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16210 / R16210C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16211 / R16211C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16212 / R16212C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16213 / R16213C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16214 / R16214C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16215 / R16215C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16216 / R16216C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16217 / R16217C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16218 / R16218C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16219 / R16219C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16220 / R16220C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16221 / R16221C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16222 / R16222C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16223 / R16223C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16224 / R16224C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16225 / R16225C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16226 / R16226C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16227 / R16227C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16228 / R16228C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16229 / R16229C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16230 / R16230C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16231 / R16231C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16232 / R16232C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16233 / R16233C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16234 / R16234C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16235 / R16235C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16236 / R16236C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16237 / R16237C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16238 / R16238C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16239 / R16239C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16240 / R16240C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16241 / R16241C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16242 / R16242C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16243 / R16243C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16244 / R16244C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16245 / R16245C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16246 / R16246C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16247 / R16247C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16248 / R16248C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16249 / R16249C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16250 / R16250C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16251 / R16251C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16252 / R16252C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16253 / R16253C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16254 / R16254C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16255 / R16255C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16256 / R16256C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16257 / R16257C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16258 / R16258C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16259 / R16259C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16260 / R16260C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16261 / R16261C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16262 / R16262C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16263 / R16263C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16264 / R16264C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16265 / R16265C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16266 / R16266C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16267 / R16267C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16268 / R16268C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16269 / R16269C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16270 / R16270C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16271 / R16271C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16272 / R16272C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16273 / R16273C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16274 / R16274C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16275 / R16275C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16276 / R16276C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16277 / R16277C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16278 / R16278C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16279 / R16279C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16280 / R16280C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16281 / R16281C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16282 / R16282C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16283 / R16283C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16284 / R16284C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16285 / R16285C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16286 / R16286C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16287 / R16287C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16288 / R16288C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16289 / R16289C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16290 / R16290C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16291 / R16291C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16292 / R16292C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16293 / R16293C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16294 / R16294C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16295 / R16295C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16296 / R16296C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16297 / R16297C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16298 / R16298C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16299 / R16299C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16300 / R16300C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16301 / R16301C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16302 / R16302C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16303 / R16303C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16304 / R16304C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16305 / R16305C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16306 / R16306C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16307 / R16307C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16308 / R16308C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16309 / R16309C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16310 / R16310C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16311 / R16311C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16312 / R16312C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16313 / R16313C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16314 / R16314C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16315 / R16315C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16316 / R16316C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16317 / R16317C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16318 / R16318C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16319 / R16319C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16320 / R16320C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16321 / R16321C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16322 / R16322C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16323 / R16323C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16324 / R16324C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16325 / R16325C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16326 / R16326C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16327 / R16327C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16328 / R16328C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16329 / R16329C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16330 / R16330C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16331 / R16331C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16332 / R16332C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16333 / R16333C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16334 / R16334C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16335 / R16335C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16336 / R16336C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16337 / R16337C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16338 / R16338C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16339 / R16339C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16340 / R16340C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16341 / R16341C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16342 / R16342C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16343 / R16343C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16344 / R16344C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16345 / R16345C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16346 / R16346C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16347 / R16347C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16348 / R16348C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16349 / R16349C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16350 / R16350C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16351 / R16351C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16352 / R16352C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16353 / R16353C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16354 / R16354C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16355 / R16355C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16356 / R16356C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16357 / R16357C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16358 / R16358C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16359 / R16359C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16360 / R16360C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16361 / R16361C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16362 / R16362C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16363 / R16363C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16364 / R16364C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16365 / R16365C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16366 / R16366C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16367 / R16367C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16368 / R16368C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16369 / R16369C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16370 / R16370C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16371 / R16371C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16372 / R16372C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16373 / R16373C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16374 / R16374C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16375 / R16375C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16376 / R16376C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16377 / R16377C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16378 / R16378C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16379 / R16379C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16380 / R16380C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16381 / R16381C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16382 / R16382C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16383 / R16383C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16384 / R16384C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16385 / R16385C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16386 / R16386C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16387 / R16387C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16388 / R16388C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16389 / R16389C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16390 / R16390C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16391 / R16391C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16392 / R16392C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16393 / R16393C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16394 / R16394C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16395 / R16395C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16396 / R16396C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16397 / R16397C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16398 / R16398C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16399 / R16399C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16400 / R16400C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16401 / R16401C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16402 / R16402C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16403 / R16403C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16404 / R16404C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16405 / R16405C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16406 / R16406C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16407 / R16407C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16408 / R16408C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16409 / R16409C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16410 / R16410C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16411 / R16411C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16412 / R16412C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16413 / R16413C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16414 / R16414C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16415 / R16415C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16416 / R16416C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16417 / R16417C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16418 / R16418C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16419 / R16419C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16420 / R16420C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16421 / R16421C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16422 / R16422C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16423 / R16423C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16424 / R16424C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16425 / R16425C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16426 / R16426C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16427 / R16427C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16428 / R16428C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16429 / R16429C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16430 / R16430C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16431 / R16431C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16432 / R16432C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16433 / R16433C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16434 / R16434C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16435 / R16435C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16436 / R16436C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16437 / R16437C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16438 / R16438C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16439 / R16439C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16440 / R16440C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16441 / R16441C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16442 / R16442C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16443 / R16443C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16444 / R16444C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16445 / R16445C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16446 / R16446C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16447 / R16447C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16448 / R16448C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16449 / R16449C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16450 / R16450C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16451 / R16451C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16452 / R16452C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16453 / R16453C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16454 / R16454C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16455 / R16455C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16456 / R16456C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16457 / R16457C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16458 / R16458C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16459 / R16459C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16460 / R16460C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16461 / R16461C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16462 / R16462C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16463 / R16463C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16464 / R16464C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16465 / R16465C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16466 / R16466C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16467 / R16467C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16468 / R16468C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16469 / R16469C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16470 / R16470C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16471 / R16471C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16472 / R16472C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16473 / R16473C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16474 / R16474C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16475 / R16475C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16476 / R16476C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16477 / R16477C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16478 / R16478C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16479 / R16479C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16480 / R16480C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16481 / R16481C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16482 / R16482C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16483 / R16483C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16484 / R16484C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16485 / R16485C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16486 / R16486C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16487 / R16487C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16488 / R16488C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16489 / R16489C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16490 / R16490C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16491 / R16491C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16492 / R16492C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16493 / R16493C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16494 / R16494C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16495 / R16495C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16496 / R16496C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16497 / R16497C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16498 / R16498C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16499 / R16499C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16500 / R16500C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16501 / R16501C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16502 / R16502C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16503 / R16503C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16504 / R16504C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16505 / R16505C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16506 / R16506C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16507 / R16507C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16508 / R16508C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16509 / R16509C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16510 / R16510C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16511 / R16511C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16512 / R16512C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16513 / R16513C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16514 / R16514C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16515 / R16515C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16516 / R16516C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16517 / R16517C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16518 / R16518C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16519 / R16519C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16520 / R16520C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16521 / R16521C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16522 / R16522C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16523 / R16523C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16524 / R16524C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16525 / R16525C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16526 / R16526C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16527 / R16527C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16528 / R16528C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16529 / R16529C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16530 / R16530C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16531 / R16531C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16532 / R16532C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16533 / R16533C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16534 / R16534C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16535 / R16535C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16536 / R16536C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16537 / R16537C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16538 / R16538C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16539 / R16539C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16540 / R16540C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16541 / R16541C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16542 / R16542C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16543 / R16543C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16544 / R16544C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16545 / R16545C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16546 / R16546C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16547 / R16547C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16548 / R16548C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16549 / R16549C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16550 / R16550C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16551 / R16551C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16552 / R16552C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16553 / R16553C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16554 / R16554C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16555 / R16555C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16556 / R16556C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16557 / R16557C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16558 / R16558C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16559 / R16559C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16560 / R16560C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16561 / R16561C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16562 / R16562C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16563 / R16563C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16564 / R16564C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16565 / R16565C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16566 / R16566C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16567 / R16567C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16568 / R16568C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16569 / R16569C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16570 / R16570C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16571 / R16571C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16572 / R16572C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16573 / R16573C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16574 / R16574C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16575 / R16575C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16576 / R16576C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16577 / R16577C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16578 / R16578C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16579 / R16579C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16580 / R16580C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16581 / R16581C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16582 / R16582C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16583 / R16583C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16584 / R16584C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16585 / R16585C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16586 / R16586C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16587 / R16587C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16588 / R16588C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16589 / R16589C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16590 / R16590C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16591 / R16591C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16592 / R16592C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16593 / R16593C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16594 / R16594C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16595 / R16595C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16596 / R16596C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16597 / R16597C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16598 / R16598C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16599 / R16599C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16600 / R16600C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16601 / R16601C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16602 / R16602C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16603 / R16603C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16604 / R16604C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16605 / R16605C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16606 / R16606C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16607 / R16607C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16608 / R16608C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16609 / R16609C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16610 / R16610C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16611 / R16611C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16612 / R16612C7: got 'NA'
```

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## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16613 / R16613C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16614 / R16614C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16615 / R16615C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16616 / R16616C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16617 / R16617C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16618 / R16618C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16619 / R16619C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16620 / R16620C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16621 / R16621C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16622 / R16622C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16623 / R16623C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16624 / R16624C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16625 / R16625C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16626 / R16626C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16627 / R16627C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16628 / R16628C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16629 / R16629C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16630 / R16630C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16631 / R16631C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16632 / R16632C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16633 / R16633C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16634 / R16634C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16635 / R16635C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16636 / R16636C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16637 / R16637C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16638 / R16638C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16639 / R16639C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16640 / R16640C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16641 / R16641C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16642 / R16642C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16643 / R16643C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16644 / R16644C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16645 / R16645C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16646 / R16646C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16647 / R16647C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16648 / R16648C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16649 / R16649C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16650 / R16650C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16651 / R16651C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16652 / R16652C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16653 / R16653C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16654 / R16654C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16655 / R16655C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16656 / R16656C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16657 / R16657C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16658 / R16658C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16659 / R16659C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16660 / R16660C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16661 / R16661C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16662 / R16662C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16663 / R16663C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16664 / R16664C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16665 / R16665C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16666 / R16666C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16667 / R16667C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16668 / R16668C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16669 / R16669C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16670 / R16670C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16671 / R16671C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16672 / R16672C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16673 / R16673C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16674 / R16674C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16675 / R16675C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16676 / R16676C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16677 / R16677C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16678 / R16678C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16679 / R16679C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16680 / R16680C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16681 / R16681C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16682 / R16682C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16683 / R16683C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16684 / R16684C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16685 / R16685C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16686 / R16686C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16687 / R16687C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16688 / R16688C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16689 / R16689C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16690 / R16690C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16691 / R16691C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16692 / R16692C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16693 / R16693C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16694 / R16694C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16695 / R16695C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16696 / R16696C7: got 'NA'
```

```
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16697 / R16697C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16698 / R16698C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16699 / R16699C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16700 / R16700C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16701 / R16701C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16702 / R16702C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16703 / R16703C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16704 / R16704C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16705 / R16705C7: got 'NA'
## Warning in read fun(path = enc2native(normalizePath(path)), sheet i = sheet, :
## Expecting numeric in G16706 / R16706C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16707 / R16707C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16708 / R16708C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16709 / R16709C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16710 / R16710C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16711 / R16711C7: got 'NA'
## Warning in read_fun(path = enc2native(normalizePath(path)), sheet_i = sheet, :
## Expecting numeric in G16712 / R16712C7: got 'NA'
resWtV2
## # A tibble: 16,708 x 7
##
      WBGeneID
                     baseMean log2FoldChange lfcSE stat
                                                             pvalue
                                                                         padj
##
      <chr>
                        <dbl>
                                       <dbl> <dbl> <dbl>
                                                              <dbl>
                                                                        <dbl>
## 1 WBGene00007894
                         903.
                                        6.98 0.182
                                                     34.0 3.42e-253 5.50e-249
                                        3.80 0.0933 32.1 1.36e-226 1.09e-222
## 2 WBGene00000783
                        1784.
## 3 WBGene00004999
                        5635.
                                       -4.70 0.122 -31.9 7.62e-224 4.08e-220
## 4 WBGene00019967
                                        3.83 0.0950 31.9 2.33e-223 9.37e-220
                        1581.
## 5 WBGene00007331
                                       -6.02 0.168 -31.1 2.72e-212 7.80e-209
                        2208.
                                       -5.85 0.162 -31.1 2.91e-212 7.80e-209
## 6 WBGene00019495
                        2321.
## 7 WBGene00000789
                        2672.
                                       -4.24 0.111 -31.0 5.66e-211 1.30e-207
## 8 WBGene00000786
                                       -4.59 0.128 -29.5 1.36e-191 2.72e-188
                        1060.
```

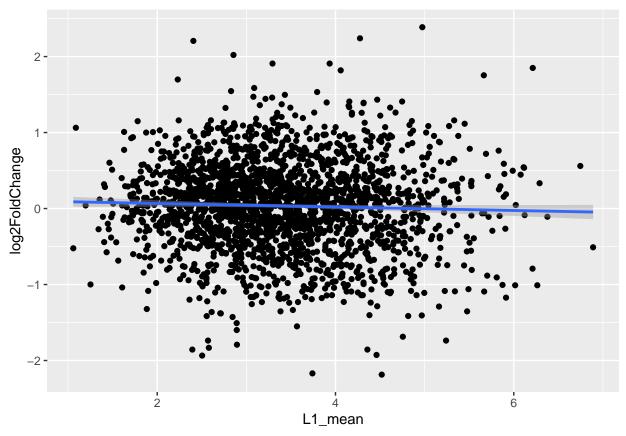
4.62 0.133

28.7 1.65e-181 2.94e-178

9 WBGene00018547

508.

`geom_smooth()` using formula 'y ~ x'



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] parallel stats4 grid stats graphics grDevices utils
```

```
[8] datasets methods
##
## other attached packages:
   [1] GenomicRanges_1.38.0 GenomeInfoDb_1.22.1
                                                   IRanges_2.20.2
                                                   lubridate_1.7.8
##
    [4] S4Vectors 0.24.4
                             BiocGenerics_0.32.0
  [7] circlize 0.4.9
                                                   dendextend 1.13.4
##
                             binom 1.1-1
## [10] RVAideMemoire 0.9-75 pheatmap 1.0.12
                                                   matrixStats 0.56.0
## [13] ComplexHeatmap_2.2.0 readxl_1.3.1
                                                   forcats 0.5.0
## [16] stringr_1.4.0
                             dplyr_0.8.5
                                                   purrr 0.3.4
## [19] readr_1.3.1
                             tidyr_1.0.3
                                                   tibble_3.0.1
## [22] ggplot2_3.3.0
                              tidyverse_1.3.0
                                                   biomaRt_2.42.1
## loaded via a namespace (and not attached):
  [1] nlme_3.1-147
                                bitops_1.0-6
                                                       fs_1.4.1
   [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
                                R6_2.4.1
## [10] utf8_1.1.4
                                                       mgcv_1.8-31
                                colorspace_1.4-1
## [13] DBI 1.1.0
                                                       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
                                xm12_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
                                                       XVector 0.26.0
## [34] pkgconfig_2.0.3
                                htmltools 0.4.0
                                                       dbplyr_1.4.3
## [37] rlang_0.4.6
                                GlobalOptions_0.1.1
                                                       rstudioapi_0.11
## [40] RSQLite_2.2.0
                                farver_2.0.3
                                                       shape_1.4.4
## [43] generics_0.0.2
                                jsonlite_1.6.1
                                                       RCurl_1.98-1.2
## [46] magrittr_1.5
                                GenomeInfoDbData_1.2.2 Matrix_1.2-18
## [49] Rcpp_1.0.4.6
                                munsell_0.5.0
                                                       fansi_0.4.1
## [52] viridis_0.5.1
                                lifecycle_0.2.0
                                                       stringi_1.4.6
## [55]
        yaml_2.2.1
                                zlibbioc_1.32.0
                                                       BiocFileCache_1.10.2
## [58] blob_1.2.1
                                crayon_1.3.4
                                                       lattice_0.20-41
## [61] splines_3.6.3
                                haven_2.2.0
                                                       hms_0.5.3
## [64] knitr 1.28
                                pillar 1.4.4
                                                       rjson 0.2.20
## [67] reprex_0.3.0
                                XML_3.99-0.3
                                                       glue_1.4.0
## [70] evaluate 0.14
                                modelr 0.1.7
                                                       png_0.1-7
## [73] vctrs_0.2.4
                                cellranger_1.1.0
                                                       gtable_0.3.0
## [76] openssl_1.4.1
                                clue_0.3-57
                                                       assertthat_0.2.1
## [79] xfun_0.13
                                broom_0.5.6
                                                       viridisLite_0.3.0
## [82] AnnotationDbi 1.48.0
                                                       cluster 2.1.0
                                memoise 1.1.0
## [85] ellipsis_0.3.0
```