ELT-2 Regulated Genes

Rtpw

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Next steps

 ${
m HIGH}$ - perform GO on up and down regulated genes LOW - elt-2 chip or promoter motifs of up and down regulated genes

Done steps

• Do Z score of row normalization, divide by the standard deviation

Improvements

Align RNA seq data to ce11 genome with more recent annotation.

Libraries

```
library(biomaRt)
library(DESeq2)
## Loading required package: S4Vectors
## Loading required package: stats4
## 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: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
##
##
## Attaching package: 'S4Vectors'
## The following object is masked from 'package:base':
##
##
       expand.grid
## Loading required package: IRanges
## Loading required package: GenomicRanges
## Loading required package: GenomeInfoDb
## Loading required package: SummarizedExperiment
## Loading required package: Biobase
## Welcome to Bioconductor
##
##
       Vignettes contain introductory material; view with
##
       'browseVignettes()'. To cite Bioconductor, see
##
       'citation("Biobase")', and for packages 'citation("pkgname")'.
## Loading required package: DelayedArray
## Loading required package: matrixStats
## Attaching package: 'matrixStats'
## The following objects are masked from 'package:Biobase':
##
       anyMissing, rowMedians
##
## Loading required package: BiocParallel
##
## Attaching package: 'DelayedArray'
## The following objects are masked from 'package:matrixStats':
##
       colMaxs, colMins, colRanges, rowMaxs, rowMins, rowRanges
##
## The following objects are masked from 'package:base':
##
       aperm, apply, rowsum
library(tidyverse)
## -- Attaching packages -----
                                      ----- tidyverse 1.3.0 --
## v ggplot2 3.3.0
                       v purrr
                                 0.3.3
## v tibble 2.1.3
                       v dplyr
                                 0.8.5
## v tidyr 1.0.2
                      v stringr 1.4.0
```

```
## v readr
            1.3.1
                       v forcats 0.5.0
## -- Conflicts ------ tidyverse conflicts() --
## x dplyr::collapse()
                         masks IRanges::collapse()
## x dplyr::combine()
                         masks Biobase::combine(), BiocGenerics::combine()
## x dplyr::count()
                         masks matrixStats::count()
## x dplyr::desc()
                         masks IRanges::desc()
## x tidyr::expand()
                         masks S4Vectors::expand()
## x dplyr::filter()
                         masks stats::filter()
## x dplyr::first()
                         masks S4Vectors::first()
## x dplyr::lag()
                         masks stats::lag()
## x ggplot2::Position() masks BiocGenerics::Position(), base::Position()
## x purrr::reduce()
                         masks GenomicRanges::reduce(), IRanges::reduce()
                         masks S4Vectors::rename()
## x dplyr::rename()
## x dplyr::select()
                         masks biomaRt::select()
                         masks DelayedArray::simplify()
## x purrr::simplify()
                         masks IRanges::slice()
## x dplyr::slice()
library(pheatmap)
library(readxl)
library(matrixStats)
Source required functions.
source("./RWC23_Functions.R")
```

Differentail Expression

Load data

```
RNAcounts <- read.csv("./01_input/Table_S1_Raw_Read_Counts.csv", header=TRUE, row.names = 1)
```

This count file contains more samples than what I want to analyze. Subset the columns to just have wt_sorted_* and elt2D_sorted_*. Also select columns that correspond to ce11 genome assembly, since this is the genome used for the ChIP-seq analysis.

```
cts <- RNAcounts %>% select(wt_sorted_1, wt_sorted_2, wt_sorted_3, wt_sorted_4, elt2D_sorted_1, elt2D_s
head(cts)
```

```
##
                   wt_sorted_1 wt_sorted_2 wt_sorted_3 wt_sorted_4 elt2D_sorted_1
## WBGene0000001
                           532
                                        462
                                                     458
                                                                  525
                                                                                  546
## WBGene00000002
                           192
                                        165
                                                                  195
                                                                                  169
                                                     185
## WBGene0000003
                           577
                                        425
                                                     649
                                                                  694
                                                                                  371
                                       1794
                                                                 1999
                                                                                 1158
## WBGene0000004
                          2111
                                                    2131
## WBGene00000005
                            11
                                          8
                                                      13
                                                                    6
                                                                                    9
## WBGene0000007
                            71
                                         82
                                                      69
                                                                   92
                                                                                   19
##
                   elt2D_sorted_2 elt2D_sorted_3 elt2D_sorted_4 elt2Delt7D_sorted_1
## WBGene0000001
                               919
                                               575
                                                                                    799
## WBGene00000002
                               226
                                               157
                                                               147
                                                                                    291
## WBGene0000003
                               557
                                               405
                                                               429
                                                                                    510
## WBGene0000004
                              1832
                                              1233
                                                              1288
                                                                                   1481
## WBGene0000005
                                                 8
                                                                10
                                                                                      3
## WBGene0000007
                                36
                                                                18
                                                                                     22
                                                15
##
                   elt2Delt7D_sorted_2 elt2Delt7D_sorted_3
                                                         482
## WBGene0000001
                                    675
```

```
## WBGene00000002
                                                                                      271
                                                                                                                                         194
## WBGene0000003
                                                                                      489
                                                                                                                                         425
## WBGene0000004
                                                                                    1304
                                                                                                                                       1347
## WBGene0000005
                                                                                           7
                                                                                                                                              1
## WBGene0000007
                                                                                         22
                                                                                                                                            13
make coldata
coldata <- data.frame(condition = c("wt", "wt", "wt", "wt", "elt2D", "
coldata
##
                                                             condition
## wt sorted 1
## wt_sorted_2
                                                                               wt.
## wt_sorted_3
                                                                               wt
## wt_sorted_4
                                                                               wt
## elt2D_sorted_1
                                                                       elt2D
## elt2D_sorted_2
                                                                       elt2D
## elt2D_sorted_3
                                                                       elt2D
## elt2D_sorted_4
                                                                       elt2D
## elt2Delt7D_sorted_1 elt2Delt7D
## elt2Delt7D_sorted_2 elt2Delt7D
## elt2Delt7D_sorted_3 elt2Delt7D
Check that column matrix and coldata match
all(rownames(coldata) == colnames(cts))
## [1] TRUE
Generate DESeqDataSet
dds <- DESeqDataSetFromMatrix(countData = cts, colData = coldata, design = ~ condition)
# add gene names
moreFeatures <- data.frame(gene_name = RNAcounts$gene_id_val, sequence_id = RNAcounts$sequence_id_list)
mcols(dds) <- DataFrame(mcols(dds), moreFeatures)</pre>
mcols(dds)
## DataFrame with 16708 rows and 2 columns
##
                                             gene_name sequence_id
##
                                                <factor>
                                                                              <factor>
                                                       aap-1 Y110A7A.10
## WBGene0000001
## WBGene00000002
                                                       aat-1
                                                                                 F27C8.1
## WBGene0000003
                                                      aat-2
                                                                                 F07C3.7
## WBGene0000004
                                                                              F52H2.2
                                                       aat-3
## WBGene0000005
                                                                        T13A10.10
                                                       aat-4
                                                             . . .
                                                                                            . . .
## WBGene00043705
                                                               NA
                                                                                             NA
## WBGene00015013
                                                               NA
                                                                                              NA
## WBGene00008743
                                                               NA
                                                                                              NA
## WBGene00235114
                                                               NA
                                                                                              NA
## WBGene00077643
                                                               NA
                                                                                              NA
```

Tell DESeq which samples are "control" and which are "control" vs "treatment". This sets up the fold change comparison manually rather than letting the alphabetical determination of factor levels.

with this step: logfoldchange(elt2D/wt)

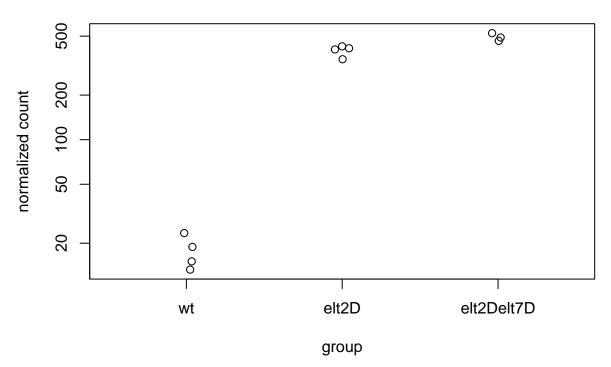
```
dds$condition <- factor(dds$condition, levels = c("wt", "elt2D", "elt2Delt7D"))</pre>
Perform differential expression analysis
dds <- DESeq(dds)
## estimating size factors
## estimating dispersions
## gene-wise dispersion estimates
## mean-dispersion relationship
## final dispersion estimates
## fitting model and testing
res <- results(dds)
# Convert res to dataframe
res.df <- as.data.frame(res)</pre>
# Export the results table
#write.csv(res.df, file = "./02_DESeq2/200218_L1_wt_vs_elt2D_results.csv")
# Print results table information
head(res)
## log2 fold change (MLE): condition elt2Delt7D vs wt
## Wald test p-value: condition elt2Delt7D vs wt
## DataFrame with 6 rows and 6 columns
##
                          baseMean
                                       log2FoldChange
                                                                   lfcSE
##
                         <numeric>
                                            <numeric>
                                                               <numeric>
## WBGene00000001 591.515264995531 0.374766904987348 0.100832250987597
## WBGene00000002 196.941946891564 0.431645564160724 0.122403401331081
## WBGene00000003 499.031409070873 -0.309270596061639 0.142658833176119
## WBGene00000004 1597.07886131967 -0.542369069007949 0.105519396720516
## WBGene00000005 7.82653928628189 -1.41145497345249 0.629860204886353
## WBGene00000007 41.2854757245755
                                     -2.0673345543798 0.274722012968266
##
                                                  pvalue
                                                                          padj
##
                                                                     <numeric>
                          <numeric>
                                               <numeric>
## WBGene00000001 3.71673647386337 0.000201812748989876 0.000660611216589531
## WBGene00000002 3.52641805265847 0.000421221497079934 0.00129548701082756
## WBGene00000003 -2.16790358631232
                                     0.0301660229459714
                                                          0.0605533480446432
## WBGene00000004 -5.13999402824958 2.74747200542808e-07 1.4149353669423e-06
## WBGene00000005 -2.24090196920309 0.0250324256740502 0.0515377102412202
## WBGene00000007 -7.52518712295034 5.26448735432883e-14 5.66900376974855e-13
summary(res)
##
## out of 16707 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)
                     : 4609, 28%
## LFC < 0 (down)
                     : 4404, 26%
## outliers [1]
                     : 16, 0.096%
## low counts [2]
                     : 0, 0%
```

```
## (mean count < 0)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
Perform vst and rlog transformation of read counts
vsd <- vst(dds)
rld <- rlog(dds)</pre>
```

Explore Differential Expression

plotCounts(dds, gene = "WBGene00001598")

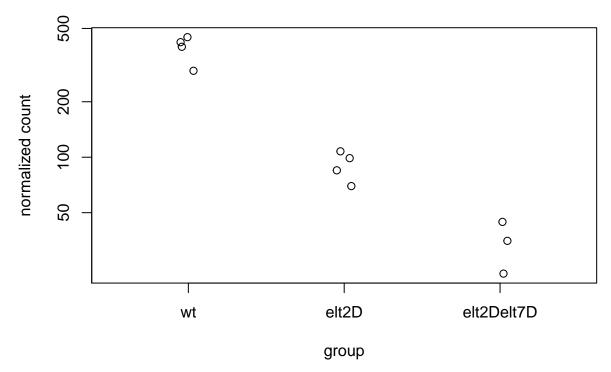
WBGene00001598



See if elt-2 is depleted It is depleted

plotCounts(dds, gene = "WBGene00001250")

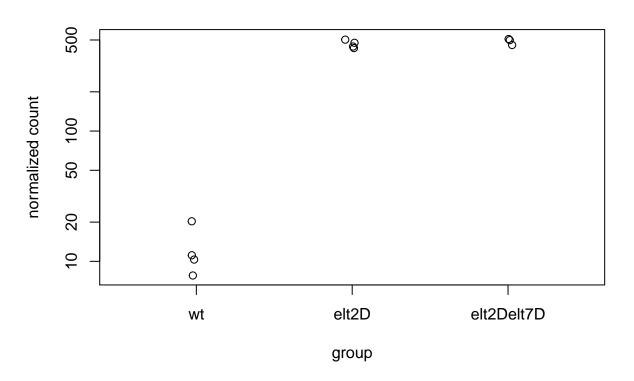
WBGene00001250



See if pgl-1 is enriched pgl-1 = WBGene00003992 Looks like it is enriched.

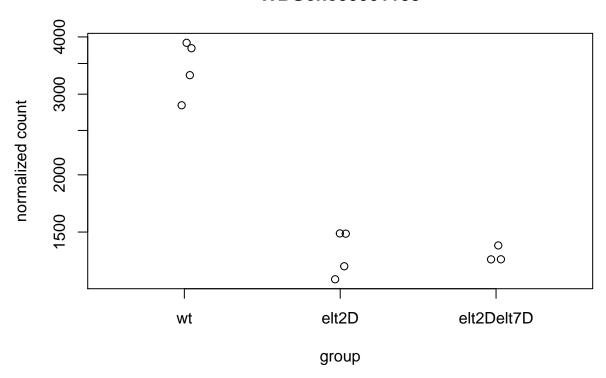
plotCounts(dds, gene = "WBGene00003992")

WBGene00003992



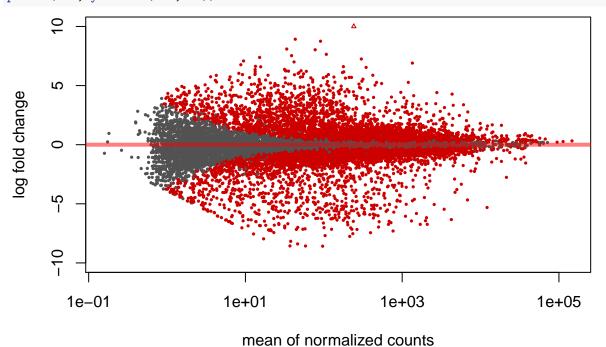
plotCounts(dds, gene = "WBGene00001188")

WBGene00001188



Make an MA plot for all the data.

plotMA(res, ylim = c(-10, 10))



Make a heatmap of differentially expressed genes.

Use variance of rlog transformed read counts to filter the data set for genes that are actually changing.

```
select <- rowVars(assay(rld)) > 0.5
rowNormalized <- assay(rld)-rowMeans(assay(rld))</pre>
pheatmap(rowNormalized[select, ], cluster_cols = FALSE, cluster_rows = TRUE, show_rownames = FALSE)
                                                                                                                                      3
                                                                                                                                      2
                                                                                                                                      1
                                                                                                                                      0
                                                                                                                                      -1
                                   wt_sorted_3
                                            wt_sorted_4
                             wt_sorted_2
                   wt_sorted_'
                                                                                                                         elt2Delt7D_sorted_3
                                                                                elt2D_sorted_
                                                                                                     elt2Delt7D_sorted_
                                                                                                               elt2Delt7D_sorted_2
                                                           elt2D_sorted_
                                                                      elt2D_sorted_
                                                                                          elt2D_sorted_4
```

Determine ELT-2 regulated TFs

```
Load in datasets
```

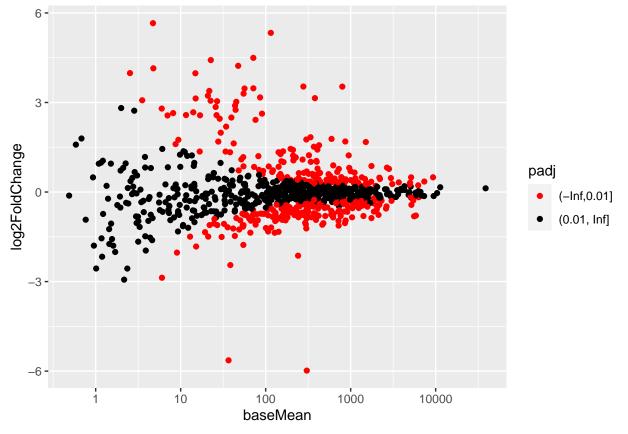
```
res.df <- read.csv(file = "./02_DESeq2/200218_L1_wt_vs_elt2D_results.csv", header = TRUE, sep = ",", ro
res.df <- rownames_to_column(res.df, var = "WBGeneID")
wTF3.0 <- read.delim("./TF3-0_namesonly.txt", header = TRUE, sep = "\t") %>% select(Sequence.name, Publ
Subset elt-2(-) differentially expressed genes for transcription factors with wTF3.0 dataset.
elt2_responding_TF <- merge(wTF3.0, res.df, by.x = "WBGeneID", by.y = "WBGeneID")
dim(elt2_responding_TF)
```

```
## [1] 854 10
head(elt2_responding_TF)
```

```
##
           WBGeneID Sequence.name Public_name
                                                            DBD
                                                                  baseMean
## 1 WBGene00000095
                         C25A1.11
                                                           bHLH 1118.11640
                                         aha-1
## 2 WBGene00000096
                          C41G7.5
                                         ahr-1
                                                           bHLH
                                                                 302.50133
## 3 WBGene00000220
                          K08F8.2
                                         atf-2 bZIP - 2 domains
                                                                   19.68263
## 4 WBGene00000221
                         T04C10.4
                                         atf-5
                                                           bZIP 2306.33631
## 5 WBGene00000222
                          F45E6.2
                                         atf-6
                                                                 699.86423
                                                           bZIP
## 6 WBGene00000223
                          C07G2.2
                                                           bZIP 4850.15422
                                         atf-7
##
     log2FoldChange
                         lfcSE
                                      stat
                                                 pvalue
                                                                padj
## 1
        -0.26323378 0.06830782 -3.8536404 1.163745e-04 3.407293e-04
## 2
        -0.58487711 0.11438142 -5.1133927 3.164235e-07 1.276946e-06
        -0.04881475 0.35069274 -0.1391952 8.892959e-01 9.258111e-01
        -0.28854464 0.07890305 -3.6569519 2.552323e-04 7.061427e-04
## 4
         0.07558784 0.07789255 0.9704117 3.318413e-01 4.374642e-01
## 5
        -0.04463394 0.05621404 -0.7939999 4.271955e-01 5.337304e-01
## 6
```

Make an MA plot of differentially expressed transcription factors.

```
threshold = 0.01
ggplot(data = elt2_responding_TF) +
  geom_point(aes(x = baseMean, y = log2FoldChange, colour = cut(padj, c(-Inf, threshold, +Inf)))) +
  scale_colour_manual(name = "padj", values = c("red", "black")
  ) +
  scale_x_log10()
```



Separate dataset into activated and repressed TFs.

```
elt2_activated_TF <- elt2_responding_TF %>% filter(log2FoldChange <= 0, padj <= threshold)
elt2_repressed_TF <- elt2_responding_TF %>% filter(log2FoldChange >= 0, padj <= threshold)</pre>
```

Make heatmap of differentially expressed TFs.

```
nameselect <- rownames(assay(rld)) %in% wTF3.0$WBGeneID
nameMatrix <- assay(rld)[nameselect,]</pre>
varselect <- rowVars(nameMatrix) > 0.1
namevarMatrix <- nameMatrix[varselect, ]</pre>
namevarRowNormalized <- namevarMatrix - rowMeans(namevarMatrix)</pre>
pheatmap(namevarRowNormalized, cluster_cols = FALSE, cluster_rows = TRUE, show_rownames = FALSE, border
                                                                                                            2
                                                                                                            1
                                                                                                            0
               wt_sorted_`
                                                                                                  elt2Delt7D_sorted_3
                       wt_sorted_2
                               wt_sorted_
                                       wt_sorted_
                                                elt2D_sorted_1
```

Replace WBGeneID with gene name in row name.

rownames(namevarRowNormalized)

```
##
     [1] "WBGene00000220" "WBGene00000431" "WBGene000004433" "WBGene00000446"
##
     [5] "WBGene00000447" "WBGene00000455" "WBGene00000458" "WBGene00000467"
     [9] "WBGene00000473" "WBGene00000474" "WBGene00000561" "WBGene00000895"
##
##
    [13] "WBGene00001174" "WBGene00001250" "WBGene00001252" "WBGene00001951"
    [17] "WBGene00001952" "WBGene00001954" "WBGene00001960" "WBGene00001973"
##
    [21] "WBGene00001977" "WBGene00002601" "WBGene00002987" "WBGene00003015"
##
##
    [25] "WBGene00003017" "WBGene00003033" "WBGene00003106" "WBGene00003114"
    [29] "WBGene00003148" "WBGene00003167" "WBGene00003228" "WBGene00003230"
##
    [33] "WBGene00003231" "WBGene00003376" "WBGene00003377" "WBGene00003511"
##
##
    [37] "WBGene00003606" "WBGene00003607" "WBGene00003633" "WBGene00003645"
    [41] "WBGene00003653" "WBGene00003657" "WBGene00003689" "WBGene00003696"
```

```
[45] "WBGene00003698" "WBGene00003702" "WBGene00003711" "WBGene00003727"
##
    [49] "WBGene00003847" "WBGene00003864" "WBGene00003865" "WBGene00003912"
##
   [53] "WBGene00003976" "WBGene00004011" "WBGene00004027" "WBGene00004078"
   [57] "WBGene00004096" "WBGene00004764" "WBGene00004786" "WBGene00005011"
##
    [61] "WBGene00006873" "WBGene00006881" "WBGene00007048" "WBGene00007058"
   [65] "WBGene00007242" "WBGene00007367" "WBGene00007749" "WBGene00007776"
##
   [69] "WBGene00008007" "WBGene00008242" "WBGene00008417" "WBGene00008830"
   [73] "WBGene00009014" "WBGene00009937" "WBGene00010215" "WBGene00011002"
##
##
    [77] "WBGene00011066" "WBGene00011100" "WBGene00011130" "WBGene00011315"
   [81] "WBGene00011376" "WBGene00011597" "WBGene00011601" "WBGene00011925"
##
   [85] "WBGene00011956" "WBGene00012005" "WBGene00012101" "WBGene00012210"
   [89] "WBGene00012435" "WBGene00012449" "WBGene00012474" "WBGene00012494"
##
   [93] "WBGene00012988" "WBGene00013270" "WBGene00013380" "WBGene00013976"
  [97] "WBGene00014253" "WBGene00015396" "WBGene00015649" "WBGene00015934"
## [101] "WBGene00016366" "WBGene00016865" "WBGene00016888" "WBGene00016930"
## [105] "WBGene00016997" "WBGene00017482" "WBGene00017651" "WBGene00017687"
## [109] "WBGene00017755" "WBGene00018099" "WBGene00018539" "WBGene00018704"
## [113] "WBGene00019327" "WBGene00019344" "WBGene00019598" "WBGene00019743"
## [117] "WBGene00019751" "WBGene00019878" "WBGene00020015" "WBGene00020555"
## [121] "WBGene00021082" "WBGene00021704" "WBGene00022060" "WBGene00022562"
## [125] "WBGene00007732" "WBGene00003688" "WBGene00011600" "WBGene00016368"
## [129] "WBGene00003648" "WBGene00004157"
paramart <- useMart("parasite_mart", dataset = "wbps_gene", host = "https://parasite.wormbase.org", por</pre>
name2id = getBM(mart = paramart,
                     filter=c("species_id_1010",
                              "biotype"),
                     value=list(species_id_1010="caelegprjna13758",
                                biotype="protein_coding"),
                     attributes = c('external_gene_id',
                                    'wbps_gene_id'))
## Cache found
head(name2id)
##
     external_gene_id
                        wbps_gene_id
## 1
              aap-1 WBGene00000001
               aat-1 WBGene00000002
## 2
## 3
               aat-2 WBGene00000003
               aat-3 WBGene00000004
## 4
## 5
               aat-4 WBGene00000005
## 6
                aat-5 WBGene00000006
```

ELT-2 Bound and Reuglated Genes

This section will integrate the L1 stage ELT-2 ChIP data analyzed by David.

Load in data.

```
elt2_peaks <- read_excel("./01_input/200331_peaksForBigBed.xlsx")

# Subset for genes bound in the L1 stage
elt2_L1_peaks <- elt2_peaks %>% select(mapped_gene, L1) %>% filter(L1 == 1) %>% select(mapped_gene)
```

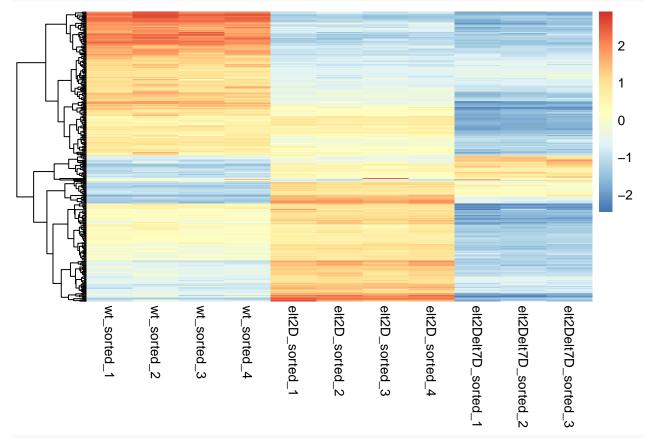
Now subset the row normalized set expression set for these genes.

Use the functions to subset and row normalize the matrix.

```
elt2_bound_matrix <- matrix_select(assay(rld), elt2_L1_peaks$mapped_gene)
elt2bound_rownormMatrix <- row_normalize_matrix(</pre>
  count_matrix = elt2_bound_matrix,
  variance_cutoff = 0.5
  )
head(elt2bound_rownormMatrix)
##
                  wt_sorted_1 wt_sorted_2 wt_sorted_3 wt_sorted_4 elt2D_sorted_1
                               -1.0652937
                                            -0.8943597
## WBGene00000022
                   -0.8776676
                                                         -1.0651520
                                                                          0.9607023
## WBGene00000136
                     1.5086891
                                 1.4044684
                                              1.5699383
                                                          1.4718552
                                                                         -0.2107053
## WBGene00000172
                    0.5423177
                                 0.6035933
                                              0.2775824
                                                          0.4495869
                                                                          0.7846391
## WBGene00000212
                    0.2258180
                                 0.6782376
                                              0.2807804
                                                          0.4314604
                                                                          0.8564958
## WBGene00000214
                     1.6529634
                                 1.9191847
                                              1.9392496
                                                          1.3315259
                                                                         -0.6143219
## WBGene00000215
                     2.0334889
                                 2.2653447
                                              2.0248544
                                                          1.9586817
                                                                         -1.3207025
##
                   elt2D_sorted_2 elt2D_sorted_3 elt2D_sorted_4 elt2Delt7D_sorted_1
## WBGene00000022
                        0.7736452
                                      0.94379989
                                                       0.7837143
                                                                           0.02098377
## WBGene00000136
                       -0.3544595
                                     -0.09764439
                                                      -0.2871333
                                                                          -1.71537290
## WBGene00000172
                        0.9472956
                                      0.66884185
                                                       0.9323174
                                                                          -1.67042064
## WBGene00000212
                        0.8710924
                                      0.96642706
                                                       0.9353808
                                                                          -1.72805622
## WBGene00000214
                       -0.2609884
                                     -0.55458218
                                                      -0.2366091
                                                                          -2.04098622
## WBGene00000215
                       -1.1089528
                                     -0.83159209
                                                      -0.9644632
                                                                          -1.45702143
##
                  elt2Delt7D_sorted_2 elt2Delt7D_sorted_3
## WBGene00000022
                            0.07090722
                                                  0.3487203
## WBGene00000136
                           -1.64382731
                                                 -1.6458084
## WBGene00000172
                           -1.81812604
                                                 -1.7176276
## WBGene00000212
                           -1.67988406
                                                 -1.8377524
## WBGene00000214
                           -1.81810338
                                                 -1.3173325
## WBGene00000215
                           -1.41765395
                                                 -1.1819836
Replace the WBGeneIDs in the row name with gene names.
elt2bound rowNormGeneNameMatrix <- id2name(elt2bound rownormMatrix)
head(elt2bound_rowNormGeneNameMatrix)
         wt_sorted_1 wt_sorted_2 wt_sorted_3 wt_sorted_4 elt2D_sorted_1
## abt-4
          -0.8776676
                      -1.0652937
                                  -0.8943597
                                                -1.0651520
                                                                 0.9607023
## amt-4
           1.5086891
                        1.4044684
                                    1.5699383
                                                 1.4718552
                                                                -0.2107053
                        0.6035933
                                                 0.4495869
## aqp-4
           0.5423177
                                    0.2775824
                                                                 0.7846391
## asm-2
           0.2258180
                        0.6782376
                                    0.2807804
                                                 0.4314604
                                                                 0.8564958
                                    1.9392496
##
  asp-1
           1.6529634
                        1.9191847
                                                 1.3315259
                                                                -0.6143219
##
   asp-2
           2.0334889
                        2.2653447
                                    2.0248544
                                                 1.9586817
                                                                -1.3207025
##
         elt2D_sorted_2 elt2D_sorted_3 elt2D_sorted_4 elt2Delt7D_sorted_1
## abt-4
              0.7736452
                             0.94379989
                                              0.7837143
                                                                  0.02098377
## amt-4
             -0.3544595
                            -0.09764439
                                             -0.2871333
                                                                 -1.71537290
## aqp-4
              0.9472956
                             0.66884185
                                              0.9323174
                                                                 -1.67042064
\#\# asm-2
              0.8710924
                             0.96642706
                                              0.9353808
                                                                 -1.72805622
## asp-1
             -0.2609884
                            -0.55458218
                                             -0.2366091
                                                                 -2.04098622
## asp-2
             -1.1089528
                            -0.83159209
                                             -0.9644632
                                                                 -1.45702143
##
         elt2Delt7D_sorted_2 elt2Delt7D_sorted_3
```

```
0.07090722
                                        0.3487203
## abt-4
## amt-4
                 -1.64382731
                                       -1.6458084
## aqp-4
                 -1.81812604
                                       -1.7176276
                 -1.67988406
                                       -1.8377524
\#\# asm-2
## asp-1
                 -1.81810338
                                       -1.3173325
## asp-2
                 -1.41765395
                                       -1.1819836
```

Now plot a heatmap of ELT-2 regulated genes.



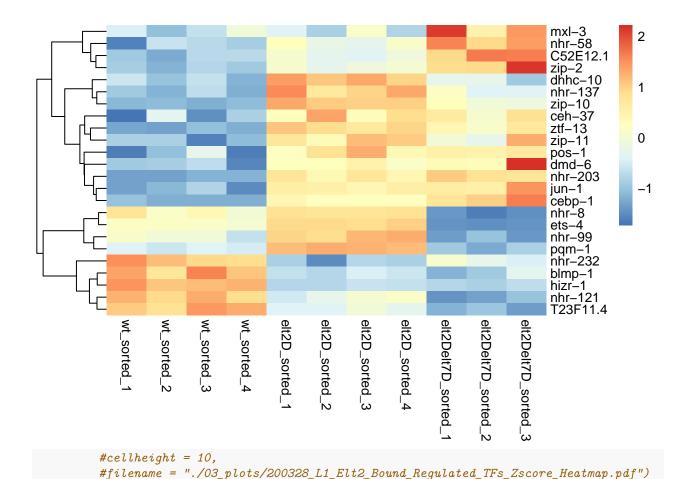
```
#cellheight = 10,
#filename = "./03_plots/200331_L1_Elt2_Elt7_Bound_Regulated_Genes_Row_Normalized_NoNames.pdf")
```

Do a similar analysis for TFs only.

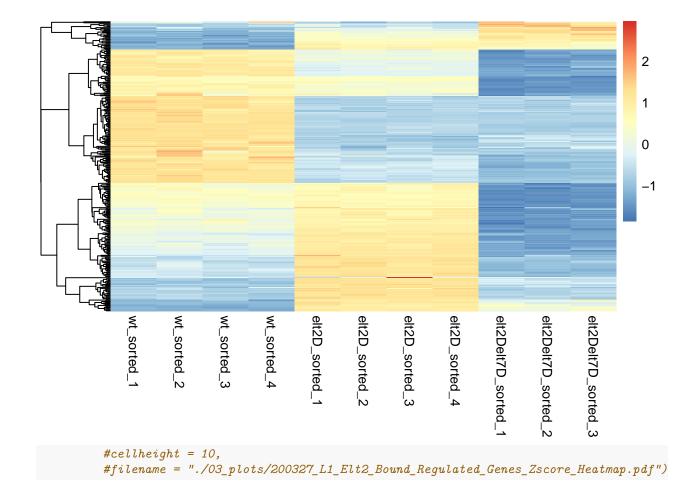
```
elt2_bound_TF_matrix <- matrix_select(count_matrix = elt2_bound_matrix, gene_subset_vector = wTF3.0$WBG
elt2_bound_TF_rowNorm_matrix <- row_normalize_matrix(
    count_matrix = elt2_bound_TF_matrix,
    variance_cutoff = 0.1
)
elt2_bound_TF_rowNorm_matrix <- id2name(elt2_bound_TF_rowNorm_matrix)</pre>
```

```
pheatmap(elt2_bound_TF_rowNorm_matrix,
               cluster_cols = FALSE,
               cluster_rows = TRUE,
               show_rownames = TRUE,
               border_color = NA)#,
                                                                                                                            mxl-3
                                                                                                                           nhr–58
C52E12.1
zip–2
                                                                                                                                                 1
                                                                                                                            cebp-1
                                                                                                                           pos-1
nhr-203
ceh-37
                                                                                                                                                 0.5
                                                                                                                                                 0
                                                                                                                           jun-1
                                                                                                                           ztf-13
dmd-6
                                                                                                                                                 -0.5
                                                                                                                           zip-11
                                                                                                                           zip-11
zip-10
pqm-1
nhr-137
dhhc-10
                                                                                                                                                 -1
                                                                                                                                                 -1.5
                                                                                                                           nhr–8
nhr–99
                                                                                                                            ets-4
                                                                                                                           hizr-1
nhr-121
nhr-232
blmp-1
T23F11.4
                   wt_sorted_1
                             wt_sorted_2
                                       wt_sorted_3
                                                                    elt2D_sorted_2
                                                                                                          elt2Delt7D_sorted_2
                                                                                                                    elt2Delt7D_sorted_3
                                                wt_sorted_4
                                                          elt2D_sorted_^
                                                                              elt2D_sorted_3
                                                                                       elt2D_sorted_
                                                                                                elt2Delt7D_sorted_
               \#cellheight = 10,
               \#filename = "./03\_plots/200331\_L1\_Elt2\_Elt7\_Bound\_Regulated\_TFs\_Row\_Normalized\_Heatmap.pdf")
```

Do it for transcription factors.



Do it for all genes.



Session info

Document session info.

```
sessionInfo()
```

```
## R version 3.6.3 (2020-02-29)
## Platform: x86 64-apple-darwin15.6.0 (64-bit)
## Running under: macOS Sierra 10.12.5
##
## Matrix products: default
          /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
                                     graphics grDevices utils
                                                                   datasets
                           stats
## [8] methods
                 base
## other attached packages:
## [1] readxl_1.3.1
                                    pheatmap_1.0.12
```

```
[3] forcats 0.5.0
                                     stringr_1.4.0
##
   [5] dplyr_0.8.5
                                     purrr_0.3.3
##
   [7] readr 1.3.1
                                     tidyr 1.0.2
## [9] tibble_2.1.3
                                     ggplot2_3.3.0
## [11] tidyverse 1.3.0
                                     DESeq2_1.26.0
## [13] SummarizedExperiment 1.16.1 DelayedArray 0.12.2
## [15] BiocParallel 1.20.1
                                    matrixStats 0.56.0
## [17] Biobase 2.46.0
                                     GenomicRanges 1.38.0
## [19] GenomeInfoDb 1.22.0
                                     IRanges 2.20.2
## [21] S4Vectors_0.24.3
                                     BiocGenerics_0.32.0
## [23] biomaRt_2.42.0
## loaded via a namespace (and not attached):
## [1] colorspace_1.4-1
                                                       XVector_0.26.0
                               htmlTable_1.13.3
## [4] base64enc_0.1-3
                                fs_1.3.2
                                                       rstudioapi_0.11
## [7] farver_2.0.3
                               bit64_0.9-7
                                                       fansi_0.4.1
## [10] AnnotationDbi_1.48.0
                                                       xm12_1.2.5
                                lubridate_1.7.4
## [13] splines 3.6.3
                                geneplotter 1.64.0
                                                       knitr 1.28
## [16] Formula_1.2-3
                                jsonlite_1.6.1
                                                       broom_0.5.5
## [19] annotate 1.64.0
                                cluster 2.1.0
                                                       dbplyr_1.4.2
## [22] png_0.1-7
                                compiler_3.6.3
                                                       httr_1.4.1
## [25] backports_1.1.5
                                assertthat 0.2.1
                                                       Matrix 1.2-18
## [28] cli_2.0.2
                                                       htmltools_0.4.0
                                acepack 1.4.1
## [31] prettyunits 1.1.1
                                tools 3.6.3
                                                       gtable 0.3.0
## [34] glue_1.3.2
                                GenomeInfoDbData_1.2.2 rappdirs_0.3.1
## [37] Rcpp_1.0.4
                                cellranger_1.1.0
                                                       vctrs_0.2.4
## [40] nlme_3.1-145
                                xfun_0.12
                                                       rvest_0.3.5
                                XML_3.99-0.3
## [43] lifecycle_0.2.0
                                                       zlibbioc_1.32.0
## [46] scales_1.1.0
                               hms_0.5.3
                                                       RColorBrewer_1.1-2
## [49] yaml_2.2.1
                                curl_4.3
                                                       memoise_1.1.0
## [52] gridExtra_2.3
                                rpart_4.1-15
                                                       latticeExtra_0.6-29
## [55] stringi_1.4.6
                                RSQLite_2.2.0
                                                       genefilter_1.68.0
## [58] checkmate_2.0.0
                                rlang_0.4.5
                                                       pkgconfig_2.0.3
## [61] bitops_1.0-6
                                                       lattice_0.20-40
                                evaluate_0.14
## [64] labeling 0.3
                               htmlwidgets_1.5.1
                                                       bit 1.1-15.2
## [67] tidyselect_1.0.0
                                                       R6_2.4.1
                               magrittr_1.5
## [70] generics 0.0.2
                               Hmisc 4.3-1
                                                       DBI 1.1.0
## [73] withr_2.1.2
                               pillar_1.4.3
                                                       haven_2.2.0
## [76] foreign_0.8-76
                                survival_3.1-11
                                                       RCurl_1.98-1.1
## [79] nnet_7.3-13
                               modelr_0.1.6
                                                       crayon_1.3.4
                                                       jpeg_0.1-8.1
## [82] BiocFileCache 1.10.2
                               rmarkdown 2.1
## [85]
       progress 1.2.2
                                locfit_1.5-9.1
                                                       grid 3.6.3
       data.table 1.12.8
## [88]
                                blob 1.2.1
                                                       reprex 0.3.0
                                xtable_1.8-4
## [91] digest_0.6.25
                                                       openssl_1.4.1
## [94] munsell_0.5.0
                                askpass_1.1
```