annotate_ALL_cryptic_introns

Figure 2B three_database_info_all_junctions.tsv

Requires simplified annotation of introns and exons as produced by leafcutter/leafviz/gtf2leafcutter.pl from gtf files for each annotation.

```
library(tidyr)
library(ggplot2)
library(dplyr)
library(ggrepel)
here::i_am("R/12_annotate_ALL_cryptic_introns.Rmd")
library(here)
knitr::opts_chunk$set(dev = "pdf",
                      dpi = 300,
                      fig.path="plots/annotate_nonsig_cryptic_plots/")
```

```
leaf_junctions = read.delim(here("31_leafcutter","with_donor_info_leafcutter.txt"))
leaf_junctions = mutate(leaf_junctions, strand = gsub(".*_","", intron),
                   start = as.integer(gsub(":.*","", intron_coords)),
                   end = as.integer(gsub(".*:","", intron_coords)),
                   genes_in_cluster = if_else(is.na(genes), "Unknown",genes))
dim(leaf_junctions)
```

```
## [1] 132587     20
```

```
summary(leaf_junctions$p.adjust < 0.05 & abs(leaf_junctions$deltapsi)>0.1)
```

```
##    Mode   FALSE    TRUE
## logical  131810     777
```

```
calc_mode = function(v){
    freq = table(v)
    as.numeric(names(freq)[which.max(freq)])
}
```

Add annotation information from ensembl

```
INDEX = "/projects/imb-pkbphil/sp/annotations/ensembl98/leafcutter_index/"
introns = read.delim(here("annotations/gencode", "gencode.v32_all_introns.bed.gz"), header = F,
                          col.names=c("chr","start","end","gene_name","gene_id","strand",
                                      "transcript_id","intron_number","biotype","annotation"))

dim(introns)
```

```
## [1] 1145034      10
```

```
ens_trans = merge(leaf_junctions, introns, by=c("chr","start","end","strand"))

ens_dist = group_by(ens_trans,  chr, start, end, strand,cluster_id,deltapsi, p.adjust, ) %>%
    summarise(transcript_ids = paste(unique(transcript_id), collapse=","),
            min_intron_number = min(intron_number),
                mode_intron_number = calc_mode(intron_number),
                gene = paste(unique(gene_name), collapse=","),
            biotype=paste(unique(biotype), collapse=","),
            genes_in_cluster =paste(unique(genes_in_cluster), collapse=",") )
```

```
## `summarise()` has grouped output by 'chr', 'start', 'end', 'strand',
## 'cluster_id', 'deltapsi'. You can override using the `.groups` argument.
```

```
ens_dist = arrange(ens_dist, p.adjust) #89k of 132k are from ensembl junctions
head(ens_dist); nrow(ens_dist)
```

```
## # A tibble: 6 x 13
## # Groups:   chr, start, end, strand, cluster_id, deltapsi [6]
##   chr      start      end strand cluster_id   deltapsi  p.adjust transcript_ids
##   <chr>    <int>    <int> <chr>  <chr>           <dbl>     <dbl> <chr>
## 1 chr7  43648652 43650493 -      clu_35616_- -0.0141   2.19e-123 ENST0000031056~
## 2 chr7  43648652 43650612 -      clu_35616_- -0.0408   2.19e-123 ENST0000044656~
## 3 chr7  43648652 43665658 -      clu_35616_- -0.000973 2.19e-123 ENST0000043844~
## 4 chr7  43648652 43711400 -      clu_35616_- -0.000367 2.19e-123 ENST0000041579~
## 5 chr7  43648652 43729429 -      clu_35616_- -0.0682   2.19e-123 ENST0000045793~
## 6 chr7  43650712 43656033 -      clu_35616_- -0.00346  2.19e-123 ENST0000043165~
## # i 5 more variables: min_intron_number <int>, mode_intron_number <dbl>,
## #   gene <chr>, biotype <chr>, genes_in_cluster <chr>
```

```
## [1] 89495
```

```
cols_to_compare = c("chr","start","end","strand", "cluster_id", "deltapsi", "p.adjust","genes_in_cluste
cryptic= setdiff(leaf_junctions[cols_to_compare], ens_dist[cols_to_compare], )
nrow(cryptic)
```

```
## [1] 43092
```

Load refseq information

```
intron_index = read.delim(here("annotations/refseq", "refseq_2023_all_introns.bed.gz"), header = F,
                          col.names=c("chr","start","end","gene_name","gene_id","strand",
                                      "transcript_id","intron_number","biotype","annotation"))
head(intron_index)
```

```
##     chr    start      end gene_name   gene_id strand   transcript_id
## 1 chr19 58353327 58353404      A1BG gene-A1BG      - rna-NM_130786.4
## 2 chr19 58353197 58353292      A1BG gene-A1BG      - rna-NM_130786.4
```

```
## 3 chr19 58352555 58352928      A1BG gene-A1BG      - rna-NM_130786.4
## 4 chr19 58351687 58352283      A1BG gene-A1BG      - rna-NM_130786.4
## 5 chr19 58350651 58351391      A1BG gene-A1BG      - rna-NM_130786.4
## 6 chr19 58347640 58350370      A1BG gene-A1BG      - rna-NM_130786.4
##   intron_number biotype annotation
## 1             1 Unknown         NA
## 2             2 Unknown         NA
## 3             3 Unknown         NA
## 4             4 Unknown         NA
## 5             5 Unknown         NA
## 6             6 Unknown         NA
```

```
table(intron_index$chr)
```

```
##
##                    chr1  chr1_KI270706v1_random  chr1_KI270708v1_random
##                  188151                      11                       3
##  chr1_KI270711v1_random  chr1_KI270712v1_random  chr1_KI270713v1_random
##                      25                      14                      11
##  chr1_KI270714v1_random                   chr10                   chr11
##                      16                   90080                  100795
##                   chr12                   chr13                   chr14
##                  109617                   39055                   59969
## chr14_GL000009v2_random chr14_GL000194v1_random chr14_KI270722v1_random
##                      24                      23                       1
## chr14_KI270723v1_random chr14_KI270724v1_random chr14_KI270725v1_random
##                       1                       2                       2
## chr14_KI270726v1_random                   chr15 chr15_KI270727v1_random
##                       7                   70982                      55
##                   chr16 chr16_KI270728v1_random                   chr17
##                   72812                     176                  105762
## chr17_GL000205v2_random                   chr18                   chr19
##                       2                   39671                   79265
##                    chr2                   chr20                   chr21
##                  171424                   38128                   18813
##                   chr22 chr22_KI270731v1_random chr22_KI270733v1_random
##                   36821                     178                      16
##                    chr3  chr3_GL000221v1_random                    chr4
##                  134762                      83                   84238
##  chr4_GL000008v2_random                    chr5                    chr6
##                       5                   81849                   93966
##                    chr7                    chr8                    chr9
##                   84317                   74623                   87637
##  chr9_KI270718v1_random  chr9_KI270719v1_random  chr9_KI270720v1_random
##                       2                      18                      20
##        chrUn_GL000195v1        chrUn_GL000213v1        chrUn_GL000214v1
##                      60                      43                       8
##        chrUn_GL000218v1        chrUn_GL000219v1        chrUn_GL000220v1
##                      18                      11                       7
##        chrUn_GL000224v1        chrUn_KI270442v1        chrUn_KI270741v1
##                      14                       7                      11
##        chrUn_KI270742v1        chrUn_KI270743v1        chrUn_KI270744v1
##                      19                      13                      41
##        chrUn_KI270745v1        chrUn_KI270746v1        chrUn_KI270748v1
```

```
##                       14                        7                       21
##        chrUn_KI270750v1        chrUn_KI270751v1        chrUn_KI270754v1
##                        5                        8                        8
##        chrUn_KI270755v1                     chrX                     chrY
##                        1                    55702                     7149
```

```
refseq = merge(cryptic, intron_index, by=c("chr","start","end","strand"))
refseq_dist = group_by(refseq, chr, start, end, strand, deltapsi, p.adjust, cluster_id) %>%
    summarise(transcript_ids = paste(unique(transcript_id), collapse=","),
            min_intron_number = min(intron_number),
                mode_intron_number = calc_mode(intron_number),
                gene = paste(unique(gene_name), collapse=","),
            genes_in_cluster =paste(unique(genes_in_cluster), collapse=","))
```

```
## `summarise()` has grouped output by 'chr', 'start', 'end', 'strand',
## 'deltapsi', 'p.adjust'. You can override using the `.groups` argument.
```

```
nrow(refseq_dist) ##11632  are found in refseq
```

```
## [1] 11632
```

```
head(refseq_dist)
```

```
## # A tibble: 6 x 12
## # Groups:   chr, start, end, strand, deltapsi, p.adjust [6]
##   chr    start    end strand deltapsi     p.adjust cluster_id  transcript_ids
##   <chr>  <int>  <int> <chr>     <dbl>        <dbl> <chr>       <chr>
## 1 chr1   14829  14970 -      0.0207   0.321        clu_27295_- rna-NR_024540.1
## 2 chr1   24891  29321 -      0.000581 0.175        clu_27299_- rna-NR_024540.1
## 3 chr1  120932 165884 -      0.0287   0.0000000573 clu_27300_- rna-XR_001737579~
## 4 chr1  187287 187380 -      0.00567  0.758        clu_27297_- rna-NR_186787.1
## 5 chr1  195416 199837 -      0.00839  0.175        clu_27299_- rna-NR_186787.1
## 6 chr1  729804 729898 -      0.00262  0.00446      clu_27306_- rna-NR_168328.1
## # i 4 more variables: min_intron_number <int>, mode_intron_number <dbl>,
## #   gene <chr>, genes_in_cluster <chr>
```

```
table(refseq_dist$min_intron_number)#22 of these are most probably a first intron
```

```
##
##    1    2    3    4    5    6    7    8    9   10   11   12   13   14   15   16
## 4582 1778  870  598  505  448  354  296  308  252  173  186  160  139  118  122
##   17   18   19   20   21   22   23   24   25   26   27   28   29   30   31   32
##   95   68   66   50   52   64   44   31   32   31   24   19   15   11   16   17
##   33   34   35   36   37   38   39   40   41   42   43   44   46   47   48   49
##   17    6    4    6    8    4    4    3    5    3    1    5    6    2    8    2
##   50   51   52   55   56   58   59   61   62   64   65   66   67   80   82  106
##    3    2    3    1    1    4    1    1    1    1    1    1    1    1    1    1
```

```
table(refseq_dist$mode_intron_number)
```

```
##
##    1    2    3    4    5    6    7    8    9   10   11   12   13   14   15   16
## 4485 1724  867  598  529  433  371  306  314  259  192  187  178  139  129  138
##   17   18   19   20   21   22   23   24   25   26   27   28   29   30   31   32
##   94   72   82   48   50   65   47   35   33   39   24   16   16   11   17   17
##   33   34   35   36   37   38   39   40   41   42   44   45   46   47   48   49
##   18    7    4    5   10    7    5    3    6    3    5    1    6    3    4    4
##   50   51   52   55   56   58   59   62   64   65   66   67   82   98  106  109
##    4    1    4    1    1    4    1    1    2    1    1    1    1    1    1    1
```

```
refseq[refseq$gene_name == "PEMT",]
```

```
##         chr    start      end strand  cluster_id   deltapsi      p.adjust
## 11354 chr17 17577027 17577107      - clu_19605_- 0.19180926 4.360252e-104
## 11355 chr17 17577027 17591967      - clu_19605_- 0.04230864 4.360252e-104
##       genes_in_cluster gene_name   gene_id      transcript_id intron_number
## 11354             PEMT      PEMT gene-PEMT rna-XM_006721418.5             1
## 11355             PEMT      PEMT gene-PEMT rna-XM_024450532.2             1
##       biotype annotation
## 11354 Unknown         NA
## 11355 Unknown         NA
```

```
#refseq_dist[grep("155243716|144816108",refseq_dist$start),]
true_cryptic = setdiff(cryptic, refseq_dist[cols_to_compare])
nrow(true_cryptic)
```

```
## [1] 31460
```

```
#true_cryptic[grep("155243716|144816108",true_cryptic$start),]
head(true_cryptic)
```

```
##     chr     start       end strand  cluster_id   deltapsi      p.adjust
## 1  chr2 238909563 238909842      + clu_32408_+ 0.40090378  3.376605e-38
## 2  chr7  43749288  43750147      - clu_35616_- 0.11671008 2.192287e-123
## 3  chr7  43730274  43750147      - clu_35616_- 0.07215141 2.192287e-123
## 4 chr11 111844723 111845565      -  clu_2011_- 0.13417677  2.039712e-62
## 5 chr15  62570852  62589687      + clu_30508_+ 0.24346848  2.041617e-34
## 6 chr10    310092    310469      - clu_37352_- 0.14518791  5.008934e-52
##   genes_in_cluster
## 1           TWIST2
## 2             COA1
## 3             COA1
## 4  ALG9,AP001781.2
## 5             TLN2
## 6            DIP2C
```

Check FANTOM CAT database

```
intron_index = read.delim(here("annotations/fantom", "fantom_cat.lv3_robust_all_introns.bed.gz"), header=
                          col.names=c("chr","start","end","gene_name","gene_id","strand",
                                      "transcript_id","intron_number","biotype","annotation"))
head(intron_index)
```

```
##     chr    start      end         gene_name           gene_id strand
## 1 chr10 69537590 69537931 CATG00000000020.1 CATG00000000020.1      -
## 2 chr10 69536724 69537457 CATG00000000020.1 CATG00000000020.1      -
## 3 chr10 69800136 69801046 CATG00000000025.1 CATG00000000025.1      -
## 4 chr10 69800136 69801046 CATG00000000025.1 CATG00000000025.1      -
## 5 chr10 69769119 69798157 CATG00000000025.1 CATG00000000025.1      -
## 6 chr10 69800136 69801046 CATG00000000025.1 CATG00000000025.1      -
##       transcript_id intron_number biotype annotation
## 1 MICT00000043247.1             1 Unknown         NA
## 2 MICT00000043247.1             2 Unknown         NA
## 3 ENCT00000057045.1             1 Unknown         NA
## 4 MICT00000043307.1             1 Unknown         NA
## 5 MICT00000043307.1             2 Unknown         NA
## 6 MICT00000043308.1             1 Unknown         NA
```

```
table(intron_index$chr)
```

```
##
##                   chr1                  chr10                  chr11
##                 288162                 123666                 164753
##                   chr12                  chr13                  chr14
##                 173866                  60791                 100581
## chr14_GL000009v2_random                  chr15    chr15_KI270850v1_alt
##                      52                 111812                     49
##                   chr16                  chr17    chr17_KI270909v1_alt
##                 116594                 174394                     13
##                   chr18                  chr19    chr19_KI270938v1_alt
##                   52930                 143379                     10
##                   chr2                   chr20                  chr21
##                 247697                  71316                  33449
##                   chr22    chr22_KI270879v1_alt                   chr3
##                   61191                     54                 185623
##                   chr4  chr4_GL000008v2_random                   chr5
##                 127234                     24                 137231
##                   chr6                   chr7     chr7_KI270803v1_alt
##                 155684                 137108                     44
##                   chr8                   chr9                   chrM
##                 106714                 123941                     20
##        chrUn_KI270742v1                   chrX                   chrY
##                     28                  90050                   2501
```

```
fantom_cat = merge(true_cryptic, intron_index, by=c("chr","start","end","strand"))
fantom_dist = group_by(fantom_cat, chr, start,end, strand, deltapsi, p.adjust,cluster_id) %>%
    summarise(transcript_ids = paste(unique(transcript_id), collapse=","),
                min_intron_number = min(intron_number),
                mode_intron_number = calc_mode(intron_number),
                gene = paste(unique(gene_name), collapse=","),
              genes_in_cluster =paste(unique(genes_in_cluster), collapse=","))
```

```
## `summarise()` has grouped output by 'chr', 'start', 'end', 'strand',
## 'deltapsi', 'p.adjust'. You can override using the `.groups` argument.
```

```
nrow(fantom_dist) ##41 out of 73 remaining unnannoted introns are founs in fantom cat
```

```
## [1] 9629
```

```
head(fantom_dist)
```

```
## # A tibble: 6 x 12
## # Groups:   chr, start, end, strand, deltapsi, p.adjust [6]
##   chr    start    end strand deltapsi   p.adjust cluster_id  transcript_ids
##   <chr>  <int>  <int> <chr>     <dbl>      <dbl> <chr>       <chr>
## 1 chr1  743350 746695 -       0.00353 0.445      clu_27307_- MICT00000000067.1
## 2 chr1  749381 753663 -       0.0106  1          clu_27305_- MICT00000000067.1
## 3 chr1  774280 778559 -       0.0320  0.00000285 clu_27309_- MICT00000000071.1,~
## 4 chr1  774280 805799 -      -0.00671 0.00000285 clu_27309_- MICT00000000069.1
## 5 chr1  801160 805799 -       0.00145 0.00000285 clu_27309_- ENCT00000020342.1
## 6 chr1  805891 810067 -       0.00315 0.00000285 clu_27309_- FTMT20100027364.1,~
## # i 4 more variables: min_intron_number <int>, mode_intron_number <dbl>,
## #   gene <chr>, genes_in_cluster <chr>
```

```
table(fantom_dist$min_intron_number) #34 of these are most probably the first intron
```

```
##
##    1    2    3    4    5    6    7    8    9   10   11   12   13   14   15   16
## 3776 1594  965  667  519  415  297  256  201  163  120   97   82   67   75   50
##   17   18   19   20   21   22   23   24   25   26   27   28   29   30   31   32
##   42   37   22   34   17   22   12   12    9   10    8    7    5    4    5    3
##   33   34   35   36   37   38   39   40   41   42   43   47   48   50   56   57
##    5    2    1    2    1    4    2    3    1    4    1    2    1    2    2    2
##   60
##    1
```

```
table(fantom_dist$mode_intron_number)
```

```
##
##    1    2    3    4    5    6    7    8    9   10   11   12   13   14   15   16
## 3565 1517  991  682  536  435  326  258  229  178  130  114   93   79   89   60
##   17   18   19   20   21   22   23   24   25   26   27   28   29   30   31   32
##   48   41   25   33   25   33   15   13   11   17   10    9    6    4    5    5
##   33   34   35   36   37   38   39   40   41   42   45   47   48   49   50   56
##    5    2    2    3    2    4    4    5    1    6    1    2    1    1    3    2
##   57   60
##    2    1
```

So perhaps we can put this info into our bar graph

```
true_cryptic = setdiff(true_cryptic,  fantom_cat[cols_to_compare])
nrow(true_cryptic)
```

```
## [1] 21831
```

```
table(true_cryptic$verdict)
```

```
## < table of extent 0 >
```

```
colnames(true_cryptic)
```

```
## [1] "chr"              "start"            "end"              "strand"
## [5] "cluster_id"       "deltapsi"         "p.adjust"         "genes_in_cluster"
```

```
true_cryptic$transcript_ids = "Unknown"
true_cryptic$biotype = "Unknown"
true_cryptic = select(true_cryptic,chr, start, end, strand, deltapsi,p.adjust,cluster_id,transcript_ids

all_annot = bind_rows(gencode=ens_dist, refseq=refseq_dist, fantom_cat=fantom_dist, cryptic= true_crypt
head(all_annot)
```

```
## # A tibble: 6 x 14
## # Groups:   chr, start, end, strand, cluster_id, deltapsi [6]
##   annotation chr      start      end strand cluster_id   deltapsi  p.adjust
##   <chr>      <chr>    <int>    <int> <chr>  <chr>           <dbl>     <dbl>
## 1 gencode    chr7  43648652 43650493 -      clu_35616_- -0.0141   2.19e-123
## 2 gencode    chr7  43648652 43650612 -      clu_35616_- -0.0408   2.19e-123
## 3 gencode    chr7  43648652 43665658 -      clu_35616_- -0.000973 2.19e-123
## 4 gencode    chr7  43648652 43711400 -      clu_35616_- -0.000367 2.19e-123
## 5 gencode    chr7  43648652 43729429 -      clu_35616_- -0.0682   2.19e-123
## 6 gencode    chr7  43650712 43656033 -      clu_35616_- -0.00346  2.19e-123
## # i 6 more variables: transcript_ids <chr>, min_intron_number <int>,
## #   mode_intron_number <dbl>, gene <chr>, biotype <chr>, genes_in_cluster <chr>
```

```
nrow(all_annot) ;#nrow(distinct(all_annot))
```

```
## [1] 132587
```

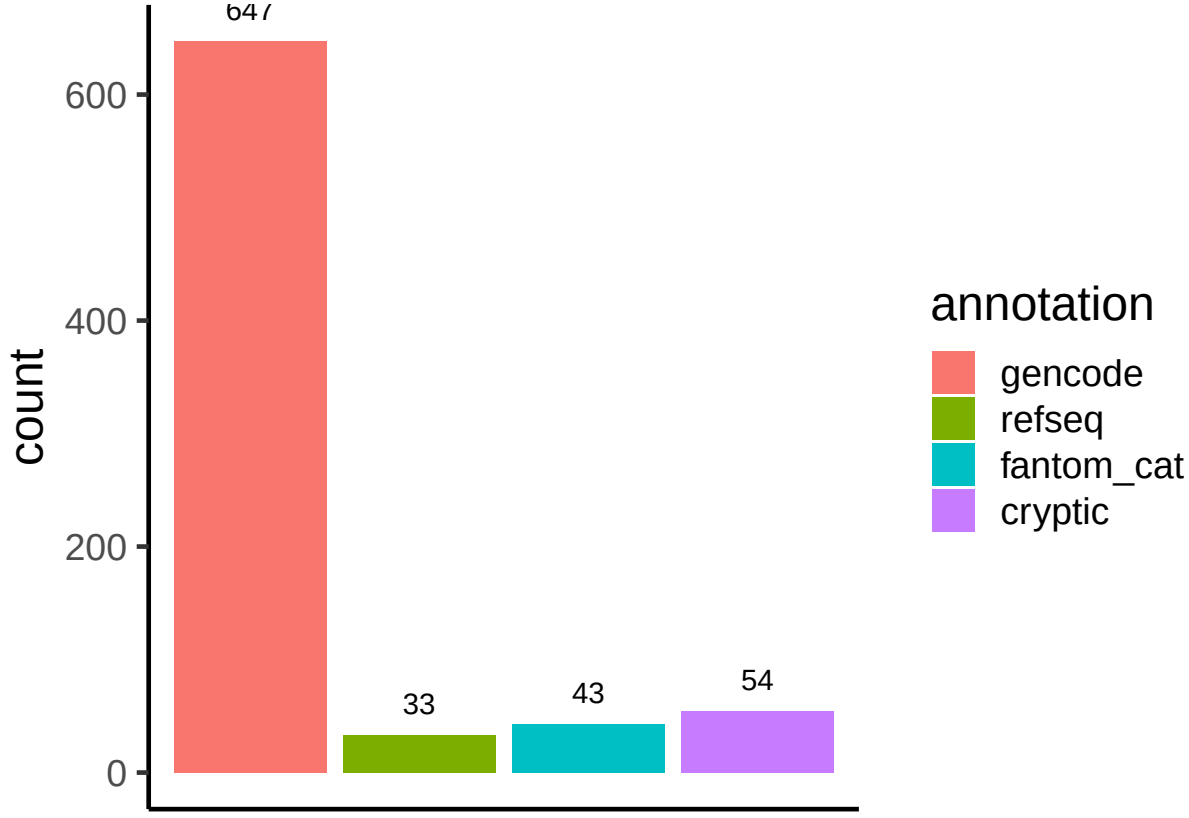
```
sig = filter(all_annot, p.adjust < 0.05 & abs(deltapsi) > 0.1)
nrow(sig)
```

```
## [1] 777
```

Plot Annotation status

```
all_annot$annotation = factor(all_annot$annotation, levels=c("gencode","refseq","fantom_cat","cryptic"))
all_annot$is_first_intron = all_annot$min_intron_number == 1

sig = filter(all_annot, p.adjust < 0.05 & abs(deltapsi) > 0.1)
ggplot(sig, aes(x=annotation, fill=annotation)) + geom_bar()  +
    theme_classic(base_size=18) + theme(axis.text.x = element_blank(), axis.ticks.x = element_blank(),
                                        panel.border = element_blank(), axis.title.x = element_blank())
  geom_text(aes(label = after_stat(count), group = annotation),
    stat="count", vjust = -1)
```



Check for cross-gene clusters

```
head(unique(all_annot$genes_in_cluster), n=20)
```

```
##  [1] "COA1"                     "PEMT"
##  [3] "PC"                       "CA5BP1,CA5B"
##  [5] "PPARG"                    "MME"
##  [7] "CITED1,AL133500.1,HDAC8" "BLOC1S1,AC009779.3,RDH5"
##  [9] "BANK1"                    "XPNPEP1"
## [11] "CD44"                     "C19orf12"
## [13] "RTN4"                     "PTK2B"
## [15] "LYRM4"                    "PEX19,AL139011.2"
## [17] "FAR2"                     "ALG9,AP001781.2"
## [19] "NCALD"                    "LPIN1"
```

```
summary(grepl( ",",all_annot$genes_in_cluster))
```

```
##    Mode   FALSE    TRUE
## logical  122196   10391
```

```
summary(grepl( ",",all_annot$genes_in_cluster) & abs(all_annot$deltapsi) > 0.1 & all_annot$p.adjust < 0
```

```
##    Mode   FALSE    TRUE
## logical  132486     101
```

```
# 100 of 693 junctions are in a cluster with another gene
# Some of these are just lowly expressed ncRNAs
# Others are known fusion / readthrough transcripts e.g. STON1-GTF2A1L
```

Save

```
head(all_annot)
```

```
## # A tibble: 6 x 15
## # Groups:   chr, start, end, strand, cluster_id, deltapsi [6]
##   annotation chr      start      end strand cluster_id   deltapsi  p.adjust
##   <fct>      <chr>    <int>    <int> <chr>  <chr>           <dbl>     <dbl>
## 1 gencode    chr7  43648652 43650493 -      clu_35616_- -0.0141   2.19e-123
## 2 gencode    chr7  43648652 43650612 -      clu_35616_- -0.0408   2.19e-123
## 3 gencode    chr7  43648652 43665658 -      clu_35616_- -0.000973 2.19e-123
## 4 gencode    chr7  43648652 43711400 -      clu_35616_- -0.000367 2.19e-123
## 5 gencode    chr7  43648652 43729429 -      clu_35616_- -0.0682   2.19e-123
## 6 gencode    chr7  43650712 43656033 -      clu_35616_- -0.00346  2.19e-123
## # i 7 more variables: transcript_ids <chr>, min_intron_number <int>,
## #   mode_intron_number <dbl>, gene <chr>, biotype <chr>,
## #   genes_in_cluster <chr>, is_first_intron <lgl>
```

```
write.table(all_annot, file=here("31_leafcutter", "three_database_info_all_junctions.tsv"),
            sep="\t", quote=F, row.names = F)
```