ChromENVEE: Chromatin ENVironment and Enhancer Expression

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Contents

Abstract

Citation

Introduction

ChromENVEE is a package developped to studying chromatin state without Hi-C data.

This package implements functions to associated genes with enhancers, define the chromatin environment of the gene from genomic data (e.g., ChromHMM output or a bed file). Several visualization functions are available to summarize the distribution of chromatin states, characterize genes associated with enhancers and also estimate the chromatin environment of genes.

```r
# Loading package
library(ChromENVEE)
```

Initialization of data

Created initial vector contains informations about chromatin state number `stateNumber`, chromatin state name `stateName` and chromatin state color `colorValue` (used for plot generation).

```r
stateNumber = c("U1","U2","U3","U4","U5","U6","U7","U8","U9","U10","U11","U12","U13","U14","U15",
"U16","U17","U18")
stateName = c("TSSA","TSSFlnk","TSSFlnkD","Tx","TxWk","EnhG","EnhG","EnhA","EnhWk","ZNFRpts","Het",
"TssBiv","EnhBiv","ReprPC","ReprPCWk","Quies","Quies","Quies")
colorValue = c("#B71C1C","#E65100","#E65100","#43A047","#1B5E20","#99FF66","#99FF66","#F5B041",
"#FFEB3B","#48C9B0","#B39DDB","#880E4F","#666633","#424949","#7B7D7D","#D0D3D4","#D0D3D4","#D0D3D4")
```

`genomeFile` is a data frame contains informations about mouse reference genome.

It is generated from bed file, in the case of this study, we used Ensembl annotation. `genomeFile` required to contains informations like chromosome (chr), gene position (start and end), strand information (strand) and gene name (gene_ENS). Score informations is suggested but not required.

```r
data(genomeFile)
```

```
#>    chr   start     end strand score             gene_ENS
#> 1 chr1 3073253 3074322      +     . ENSMUSG00000102693.1
#> 2 chr1 3102016 3102125      +     . ENSMUSG00000064842.1
#> 3 chr1 3205901 3671498      -     . ENSMUSG00000051951.5
#> 4 chr1 3252757 3253236      +     . ENSMUSG00000102851.1
#> 5 chr1 3365731 3368549      -     . ENSMUSG00000103377.1
#> 6 chr1 3375556 3377788      -     . ENSMUSG00000104017.1
```

`chromatinState` is a data frame contains informations about chromatin state.

It is generated with the output of ChromHMM tools. `chromatinState` required to contains informations like chromosome (chr), genomic regions (start and end), chromatin state information (state and state_name) and sample name (name)

```r
data(chromatinState)
```

```
#>     chr   start     end state name state_name
#> 1 chr10       0 3100000   U16   RS      Quies
#> 2 chr10 3100000 3109200   U11   RS        Het
#> 3 chr10 3109200 3110600   U12   RS     TssBiv
#> 4 chr10 3110600 3111000   U14   RS     ReprPC
#> 5 chr10 3111000 3111200   U13   RS     EnhBiv
#> 6 chr10 3111200 3117200   U12   RS     TssBiv
```

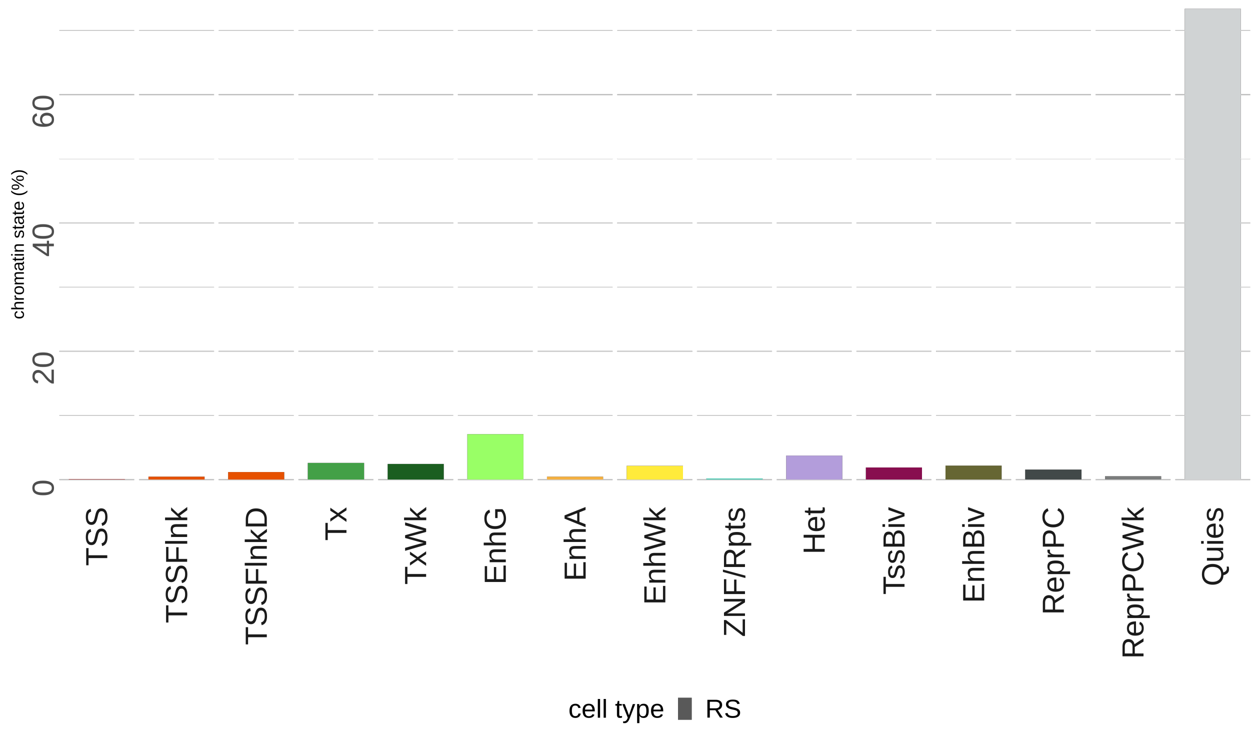
Distribution of chromatin state in the genome

We are interested to know the distribution of chromatin state in the genome.

`plotChromatinState` calculates the percentage of each chromatin state in function the length of the genome used. We obtains a data frame with the percentage of coverage for each chromatin state. It's possible to plot the result in `.png` file with the argument `plot = TRUE`. If you have a list of data frame, it's possible to merge all the data frame in merge data frame and in unique plot with `merge = TRUE` argument.

```
summary_chromatin_state = plotChromatinState(chromatinState, stateName = stateName,
stateNumber = stateNumber, merge = TRUE, plot = FALSE, color = colorValue, filename = "")
```

```
head(summary_chromatin_state)
#>              state   coverage sample_name
#> TSSA          TSSA 0.08519426          RS
#> TSSFlnk    TSSFlnk 0.45530134          RS
#> TSSFlnkD TSSFlnkD 1.18900667           RS
#> Tx              Tx 2.60257103          RS
#> TxWk          TxWk 2.44911129          RS
#> EnhG          EnhG 7.10081351          RS
```



Annotation of enhancer

We are interested to associated at each enhancer, genes regulated by the enhancer. We focused on enhancer chromatin state (in this study, we have 4 tpe of enhancer : bivalent enhancer (EnhBiv), genic enhancer (EnhG), active enhancer (EnhA) and weak enhancer (EnhWk)).

`listTableEnhancer` is a GRanges object or a list of GRanges object (producted by `GenomicRanges` package). Like chromatinState data frame, `listTableEnhancer` required gene information and chromatin state information. Sample name (sample_name) is required if you want compared enhancer annotation (see Enhnacer annotation comparison part).

```
data(listTableEnhancer)
```

```
#> GRanges object with 1979 ranges and 2 metadata columns:
#>          seqnames             ranges strand | chromatin_state
#>             <Rle>          <IRanges>  <Rle> |     <character>
#>      [1]    chr10    9164400-9164800      * |             U13
#>      [2]    chr10     9342200-9344000      * |            U13
#>      [3]    chr10   10476400-10476600      * |            U13
#>      [4]    chr10   20520200-20521000      * |            U13
#>      [5]    chr10   20952400-20952600      * |            U13
#>      ...      ...                 ...    ... .            ...
#>   [1975]     chrX 144286800-144287000      * |            U13
#>   [1976]     chrX 155128400-155129200      * |            U13
#>   [1977]     chrX 170010800-170013800      * |            U13
#>   [1978]     chrY       198400-198800      * |            U13
#>   [1979]     chrY   90786000-90788000      * |            U13
#>                  sample_name
#>                  <character>
#>      [1] RS_18_EnhBiv_H3K79me2
#>      [2] RS_18_EnhBiv_H3K79me2
#>      [3] RS_18_EnhBiv_H3K79me2
#>      [4] RS_18_EnhBiv_H3K79me2
#>      [5] RS_18_EnhBiv_H3K79me2
#>      ...                   ...
#>   [1975] RS_18_EnhBiv_H3K79me2
#>   [1976] RS_18_EnhBiv_H3K79me2
#>   [1977] RS_18_EnhBiv_H3K79me2
#>   [1978] RS_18_EnhBiv_H3K79me2
#>   [1979] RS_18_EnhBiv_H3K79me2
#>   -------
#>   seqinfo: 21 sequences from an unspecified genome; no seqlengths
```

Annotated enhancer binding to enhancer position

To estimated which gene is regulated by enhancer, we estimated that genes associated enhancer, all TSS genes in interval around enhancer. `enhancerAnnotation()` uses a `GRanges` object.

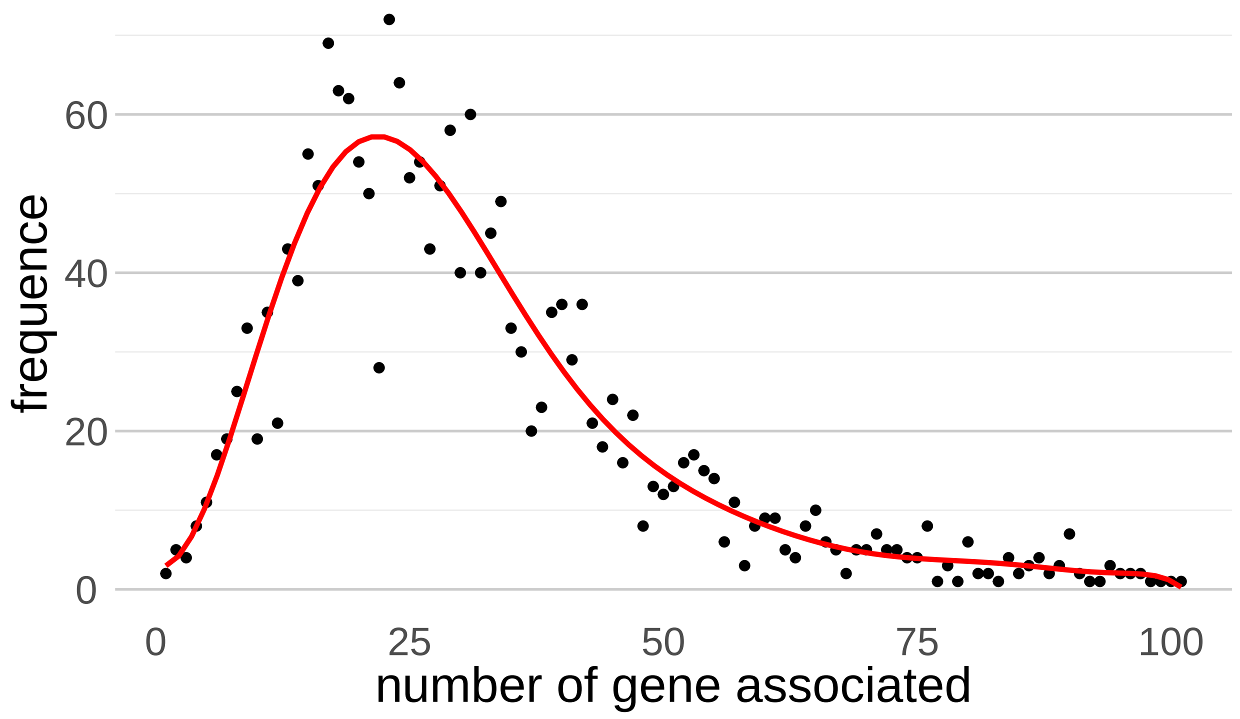
The function take few minutes to process in function the length of your enhancer table. It's possible to multithread the job with the `nCore` parameter. For each enhancer position, we obtains two informations, the distance between gene and enhancer (in bp) and the gene.

```
table_enhancer_gene = enhancerAnnotation(listTableEnhancer[[1]],genome = genomeFile,
interval = 500000, nCore = 1)
```

Number of gene associate at the enhancer

We want to know the distribution of genes associated at each enhancer.

```
plotGeneAssociation(table_enhancer_gene, all = FALSE)
```



Associated gene expression to enhancer

`geneExpression` is a data frame contains gene expression level information.

It is generated with RNAseq gene expression analysis. `geneExpression` required to contains informations like chromosome (chr), gene position (start and end), gene name (gene_ENS), strand information (strand), level of gene expression (gene_expression). Score is not required for the analysis. For gene name, you need to used the same gene name that you used to generated `genomeFile` data frame because we used the annotation to associated the couple gene-enhancer with the expression.

```
data(geneExpression)
```

```
#>                  gene_ENS   chr     start       end strand score gene_expression
#> 1  ENSMUSG00000000001.4  chr3 108107280 108146146      -     .      27.7106904
#> 2 ENSMUSG00000000028.15 chr16  18780447  18811987      -     .      23.5842993
#> 3 ENSMUSG00000000031.16  chr7 142575529 142578143      -     .       0.9386427
#> 4 ENSMUSG00000000037.16  chrX 161117193 161258213      +     .      14.4548991
#> 5 ENSMUSG00000000049.11 chr11 108343354 108414396      +     .      36.6169129
#> 6  ENSMUSG00000000056.7 chr11 121237253 121255856      +     .       5.2791187
```

We associated the level of gene expression at each gene-enhancer couple estimate with `enhancerAnnotation` function.

According to `geneExpression` data frame, it's possible that gene-enhancer couple has not expression level, in this case, we obtains NA value.

```
table_enhancer_gene_expression = enhancerExpression(table_enhancer_gene,
  geneExpressionTable = geneExpression)
```

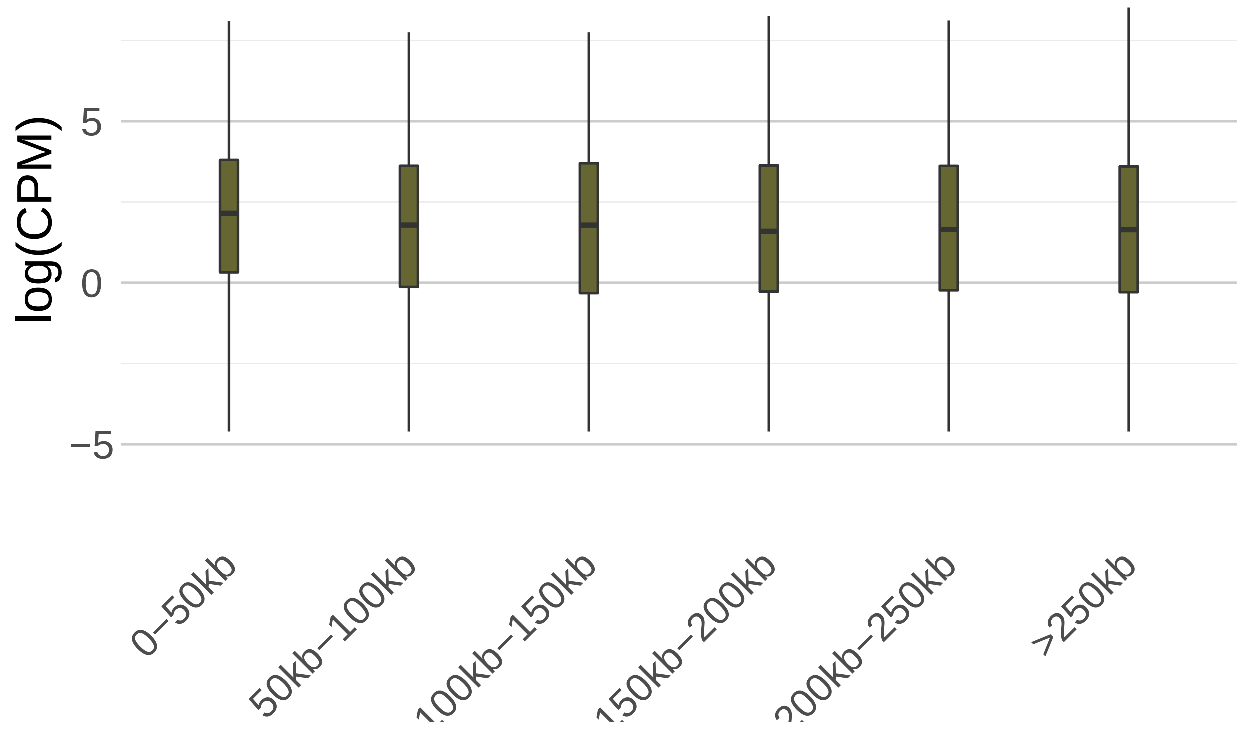
```
#> GRanges object with 6 ranges and 8 metadata columns:
#>     seqnames            ranges strand | chromatin_state           sample_name
#>        <Rle>         <IRanges>  <Rle> |     <character>           <character>
#>   1    chr10   9164400-9164800      * |             U13 RS_18_EnhBiv_H3K79me2
#>   1    chr10   9342200-9344000      * |             U13 RS_18_EnhBiv_H3K79me2
#>   1    chr10 10476400-10476600      * |             U13 RS_18_EnhBiv_H3K79me2
#>   1    chr10 20520200-20521000      * |             U13 RS_18_EnhBiv_H3K79me2
#>   1    chr10 20952400-20952600      * |             U13 RS_18_EnhBiv_H3K79me2
#>   1    chr10 21309400-21310600      * |             U13 RS_18_EnhBiv_H3K79me2
#>     start_500kb end_500kb gene_association               distance
#>       <numeric> <numeric>        <integer>            <character>
#>   1     8664400   9664800               19 451159;278330;340253..
#>   1     8842200   9844000               21 456130;480757;457563..
#>   1     9976400  10976600               20 499773;435480;392457..
#>   1    20020200  21021000               16 371729;318362;311710..
#>   1    20452400  21452600               21 227322;432632;326765..
#>   1    20809400  21810600               21 430427;356853;275607..
#>                   gene_list        gene_expression
#>                 <character>            <character>
#>   1 ENSMUSG00000111215.1.. NA;12.8456863815602;..
#>   1 ENSMUSG00000015305.6.. 12.8456863815602;2.0..
#>   1 ENSMUSG00000101621.2.. NA;NA;NA;NA;NA;NA;NA..
#>   1 ENSMUSG00000019996.1.. 102.374504394998;2.0..
#>   1 ENSMUSG00000019990.1.. 0.571438637996035;3...
#>   1 ENSMUSG00000111177.1.. NA;399.268224715743;..
#>   -------
#>   seqinfo: 21 sequences from an unspecified genome; no seqlengths
```

Profile of enhancer annotation

Distance gene-enhancer according to their expression

We generated plot to estimated the level of gene expression according to the distance between gene and enhancer.

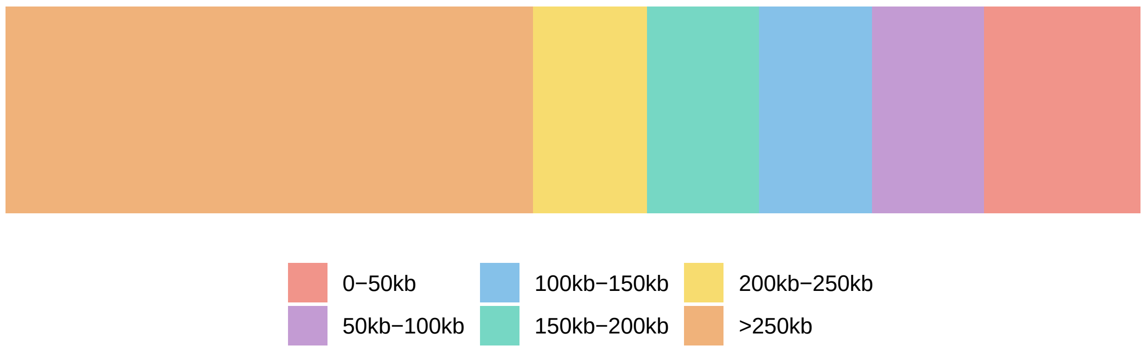
```
plotDistanceExpression(table_enhancer_gene_expression, color = colorValue, stateName = stateName,
stateNumber = stateNumber)
```



Distance gene-enhancer

We generated plot to estimated the distribution of gene according to the distance between gene and enhancer.

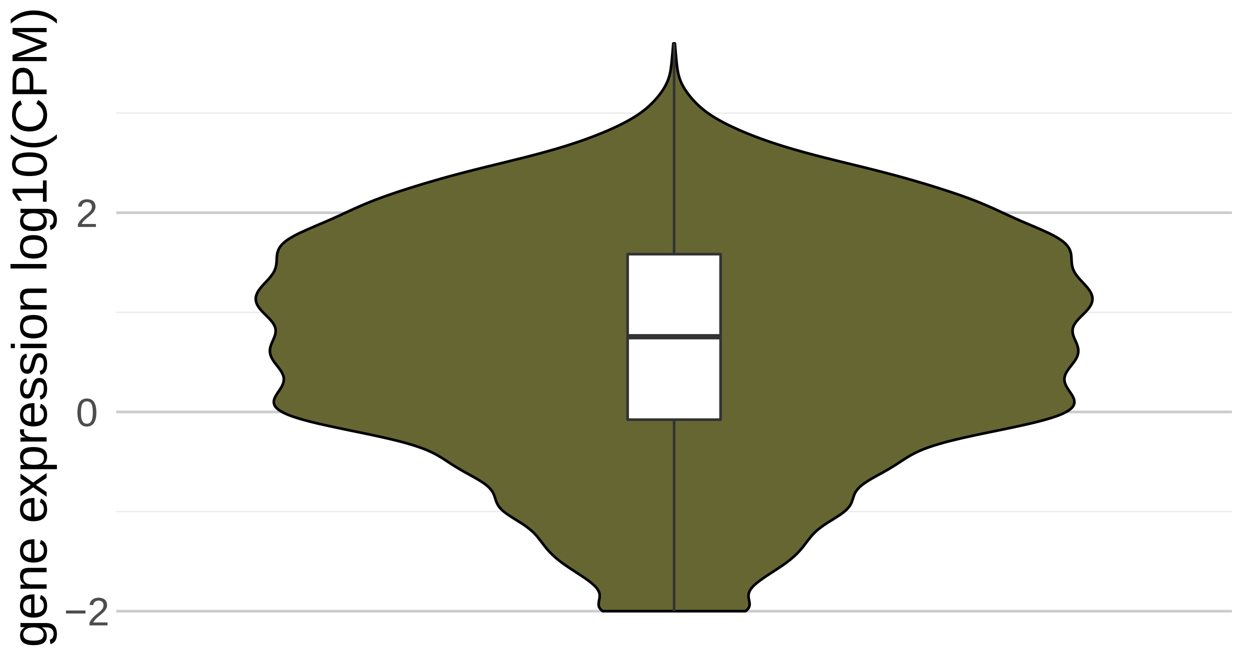
```
plotGeneDistance(table_enhancer_gene_expression)
```



Enhancer expression

We generated plot with the distribution of gene expression associated at enhancer region. It's possible to rescale plot with `scale` argument ('none','log10' and 'log2' are accepted).

```
plotEnhancerExpression(table_enhancer_gene_expression, scale = "log10", color = colorValue,
stateName = stateName, stateNumber = stateNumber, ylab = "gene expression log10(CPM)")
```



Enhancer annotation comparison

It's possible to compared different categories of enhancer. For that it's necessary to used a list of GRanges object each contains data like `listTableEnhancer` data. Contrary to individual analysis, each GRanges object in the list required sample information (sample_name).

The first step is associated gene to each enhancer using `enhancerAnnotation()` on the list of enhancer. After the gene association, we associated the gene expression using `enhancerExpression()`. In the case of this study, all enhancer categories come from same cell type, we also used the same `geneExpression` data frame.

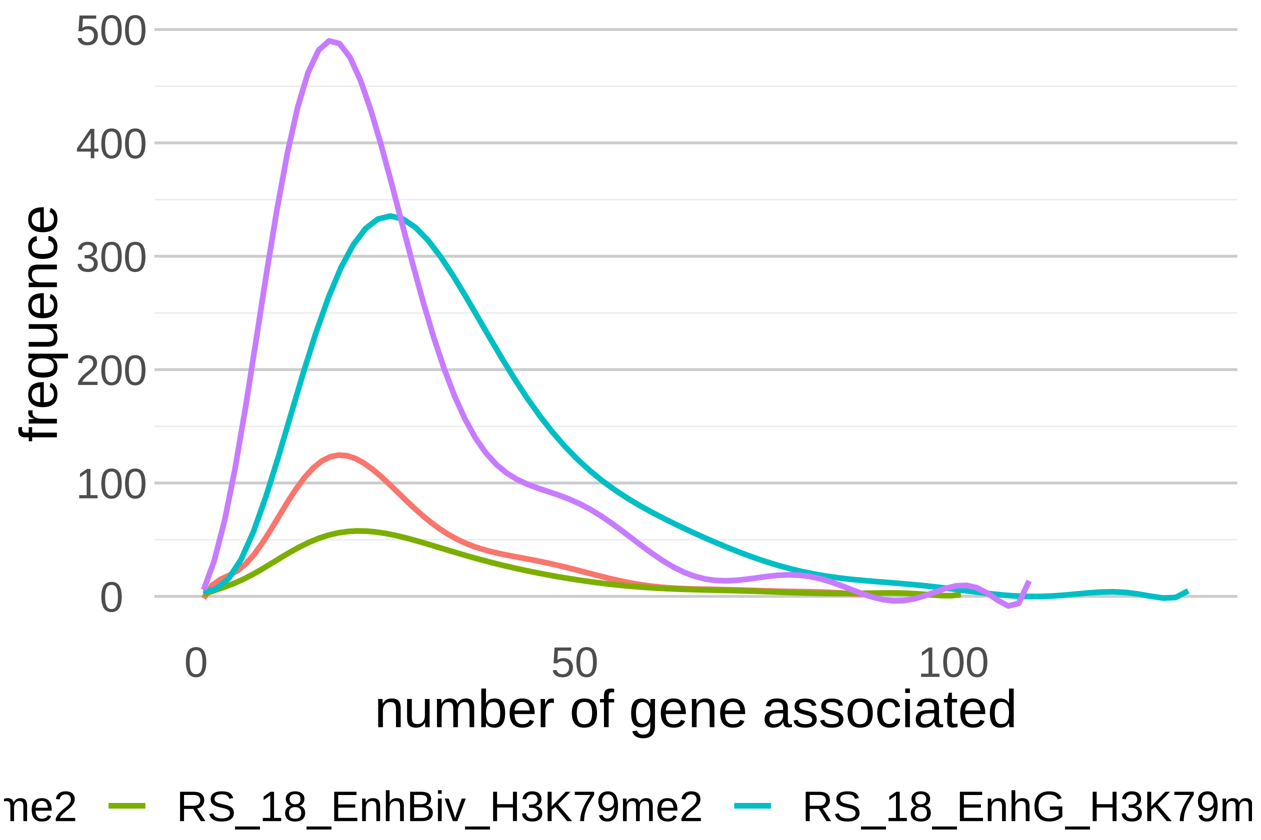
```
list_table_enhancer_gene = lapply(listTableEnhancer, enhancerAnnotation, genome = genomeFile,
interval = 500000, nCore = 1)
list_table_enhancer_gene_expression = lapply(list_table_enhancer_gene, enhancerExpression,
geneExpressionTable = geneExpression)
```

This process take many time. To reduce time, you can load `list_table_enhancer_gene_expression` data to process the next analysis. `data(list_table_enhancer_gene_expression)`

Number of gene associate at the enhancer

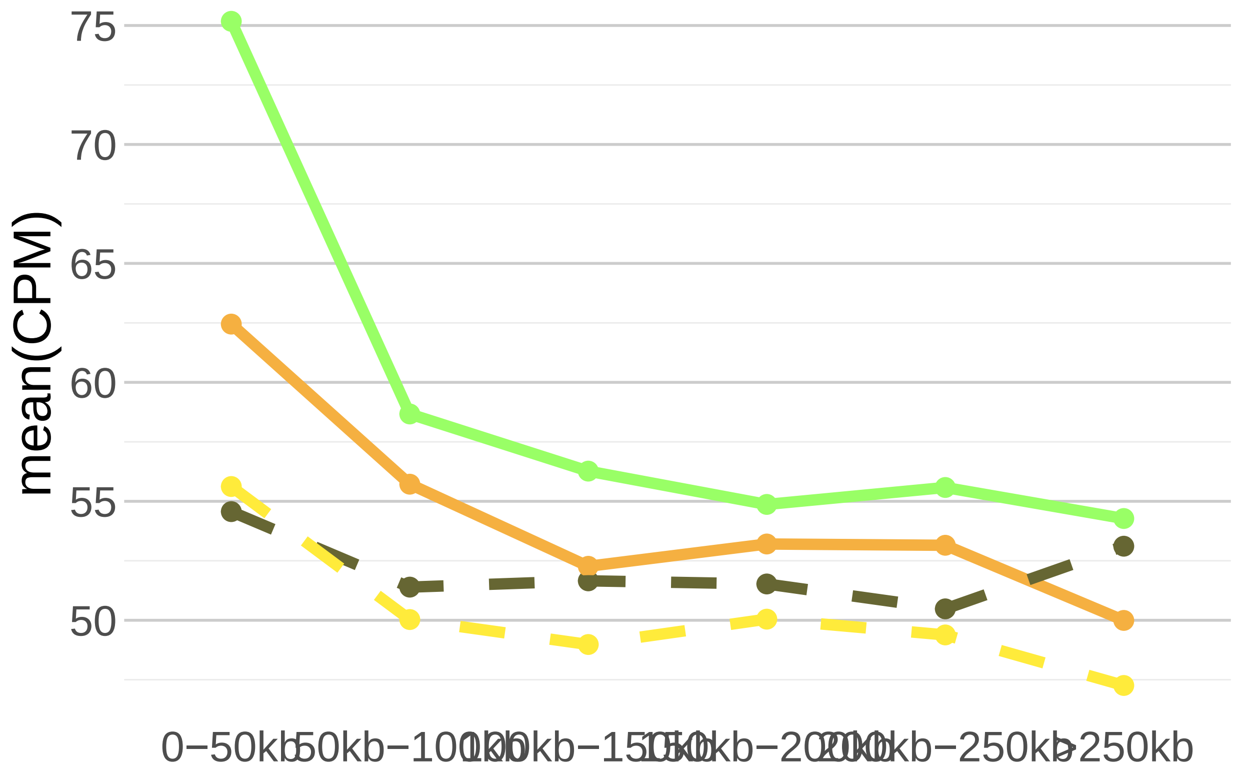
We want to know the distribution of genes associated at each enhancer. `all = TRUE` parameter is used to compiled all enhancer table in same file.

```
plotGeneAssociation(list_table_enhancer_gene_expression, all = TRUE)
```



Distance gene-enhancer in function their expression We generated plot to estimated the level of gene expression according to the distance between gene and enhancer.

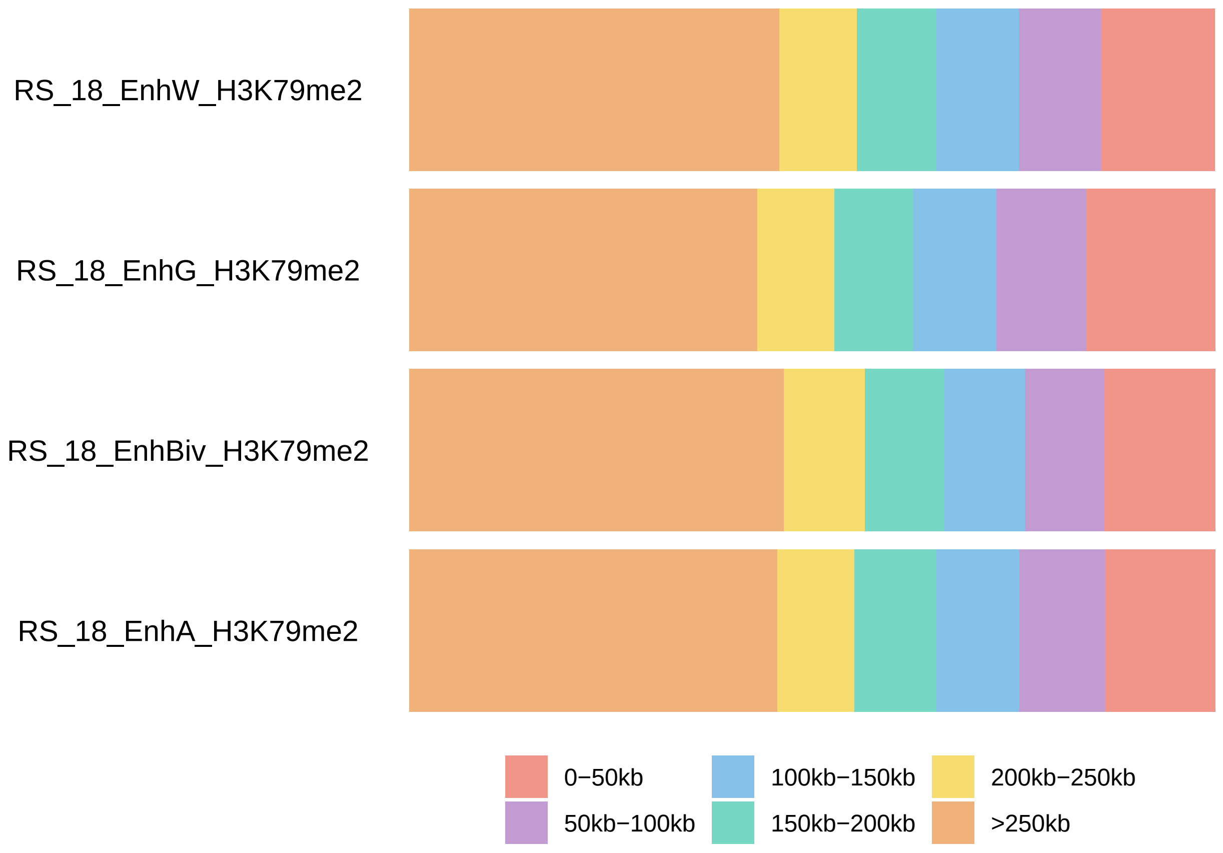
```
plotDistanceExpression(list_table_enhancer_gene_expression, color = colorValue,
stateName = stateName, stateNumber = stateNumber)
```



Distance gene-enhancer

We generated plot to estimated the distribution of gene according to the distance between gene and enhancer.

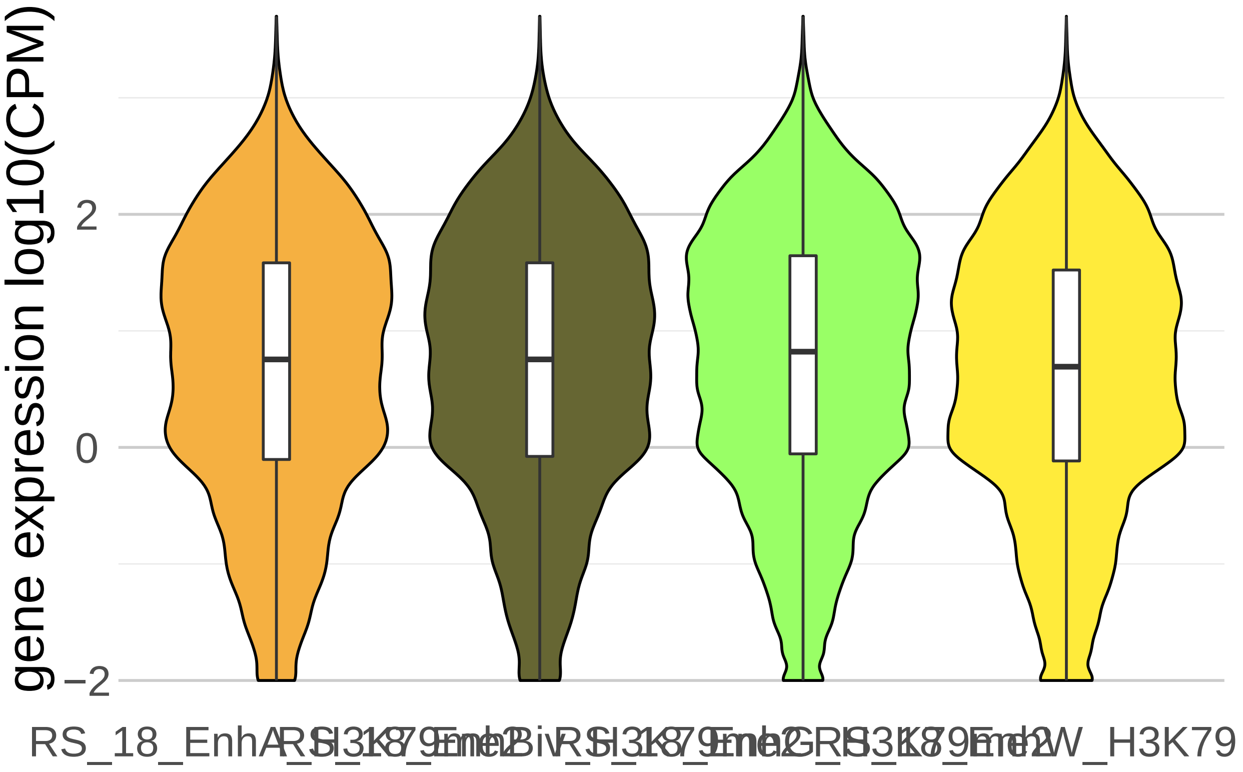
```
plotGeneDistance(list_table_enhancer_gene_expression)
```



Enhancer expression

We generated plot with the distribution of gene expression associated at enhancer region. It's possible to rescale plot with `scale` argument ('none','log10' and 'log2' are accepted).

```
plotEnhancerExpression(list_table_enhancer_gene_expression, scale = "log10", color = colorValue,
stateName = stateName, stateNumber = stateNumber, ylab = "gene expression log10(CPM)")
```



Gene environment

We are interested to studying the chromatin environment of gene.

```
stateOrderReduce = c("TSSA","TSSFlnk","TSSFlnk","Tx","Tx","EnhG","EnhG","EnhA","EnhWk",
"ZNF.Rpts","Het","TssBiv","EnhBiv","ReprPC","ReprPC","Quies","Quies","Quies")
```

```
data(geneExpression)
data(chromatinState)
```

Coverage of chromatin state in environment binding to TSS regions

To studying the environment of genes, we estimated as environment the interval (`interval` argument) around the TSS. `geneEnvironment()` may take few minutes in function the number of genes analyzed. For each gene, we obtains informations about the coverage of each chromatin state in the environment.

```
table_overlapping = geneEnvironment(geneExpression, chromatinState, stateOrder = unique(stateOrderReduc
rownames(table_overlapping) = table_overlapping$gene_ENS
```

```
#>                                            gene_ENS   chr     start       end strand
#> ENSMUSG00000000001.4   ENSMUSG00000000001.4  chr3 108107280 108146146      -
#> ENSMUSG00000000028.15 ENSMUSG00000000028.15 chr16  18780447  18811987      -
#> ENSMUSG00000000031.16 ENSMUSG00000000031.16  chr7 142575529 142578143      -
#> ENSMUSG00000000037.16 ENSMUSG00000000037.16  chrX 161117193 161258213      +
#> ENSMUSG00000000049.11 ENSMUSG00000000049.11 chr11 108343354 108414396      +
#> ENSMUSG00000000056.7   ENSMUSG00000000056.7 chr11 121237253 121255856      +
#>                       score gene_expression       TSS TSS_moins_3kb
#> ENSMUSG00000000001.4      .      27.7106904 108146146     108143146
#> ENSMUSG00000000028.15     .      23.5842993  18811987      18808987
#> ENSMUSG00000000031.16     .       0.9386427 142578143     142575143
#> ENSMUSG00000000037.16     .      14.4548991 161117193     161114193
#> ENSMUSG00000000049.11     .      36.6169129 108343354     108340354
#> ENSMUSG00000000056.7      .       5.2791187 121237253     121234253
#>                       TSS_plus_3kb       TSSA    TSSFlnk Tx      EnhG EnhA
#> ENSMUSG00000000001.4     108149146 0.00000000 0.00000000  0 0.7423333    0
#> ENSMUSG00000000028.15     18814987 0.00000000 0.06666667  0 0.6333333    0
#> ENSMUSG00000000031.16    142581143 0.00000000 0.00000000  0 0.0000000    0
#> ENSMUSG00000000037.16    161120193 0.03333333 0.40000000  0 0.0000000    0
#> ENSMUSG00000000049.11    108346354 0.00000000 0.00000000  0 0.0000000    0
#> ENSMUSG00000000056.7     121240253 0.00000000 0.06666667  0 0.6000000    0
#>                        EnhWk ZNF.Rpts Het    TssBiv EnhBiv ReprPC     Quies
#> ENSMUSG00000000001.4  0.0000        0   0 0.2576667    0.0 0.0000 0.0000000
#> ENSMUSG00000000028.15 0.0000        0   0 0.3000000    0.0 0.0000 0.0000000
#> ENSMUSG00000000031.16 0.0000        0   0 0.0000000    0.4 0.3095 0.2905000
#> ENSMUSG00000000037.16 0.1655        0   0 0.0000000    0.0 0.0000 0.4011667
#> ENSMUSG00000000049.11 0.0000        0   0 0.3000000    0.3 0.3410 0.0590000
#> ENSMUSG00000000056.7  0.0000        0   0 0.3333333    0.0 0.0000 0.0000000
```

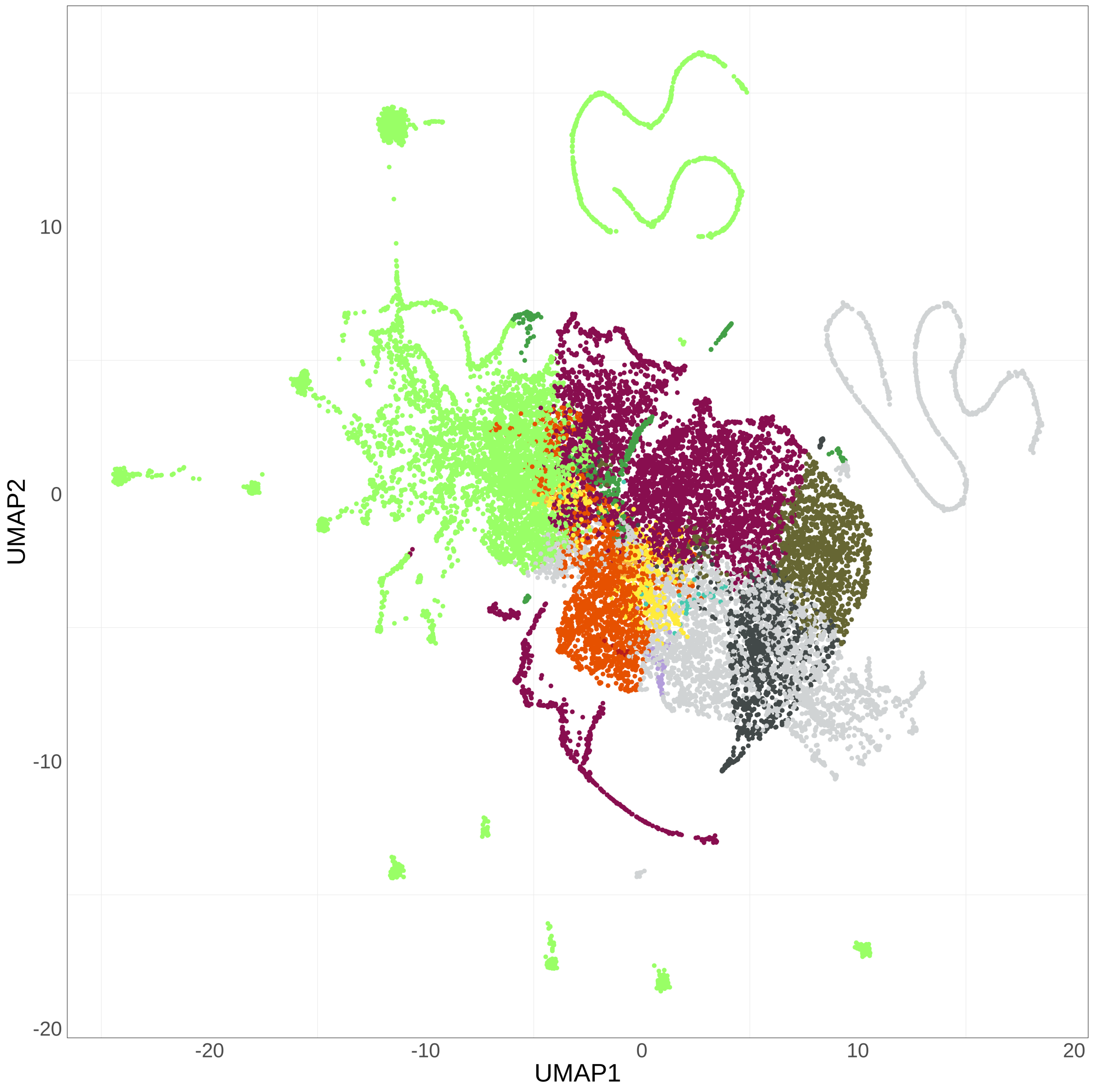
Predominant state in environment binding to TSS regions

We estimated as predominent chromatin state in the environment, the chromatin state with the higher coverage in the environment. For each gene, we use `umap` package to estimated the cluster dimension.

```
result_umap = predominentState(table_overlapping, state = unique(stateOrderReduce),
header = unique(stateOrderReduce), neighbors = 32, metric = "euclidean", dist = 0.5)
#>
#> ==> It will be take few minutes to process
```

```
#>                             TSSA    TSSFlnk Tx      EnhG EnhA  EnhWk ZNF.Rpts
#> ENSMUSG00000000001.4  0.00000000 0.00000000  0 0.7423333    0 0.0000        0
#> ENSMUSG00000000028.15 0.00000000 0.06666667  0 0.6333333    0 0.0000        0
#> ENSMUSG00000000031.16 0.00000000 0.00000000  0 0.0000000    0 0.0000        0
#> ENSMUSG00000000037.16 0.03333333 0.40000000  0 0.0000000    0 0.1655        0
#> ENSMUSG00000000049.11 0.00000000 0.00000000  0 0.0000000    0 0.0000        0
#> ENSMUSG00000000056.7  0.00000000 0.06666667  0 0.6000000    0 0.0000        0
#>                       Het    TssBiv EnhBiv ReprPC     Quies      UMAP1
#> ENSMUSG00000000001.4    0 0.2576667    0.0 0.0000 0.0000000 15.3444005
#> ENSMUSG00000000028.15   0 0.3000000    0.0 0.0000 0.0000000 11.2461522
#> ENSMUSG00000000031.16   0 0.0000000    0.4 0.3095 0.2905000  1.3368335
#> ENSMUSG00000000037.16   0 0.0000000    0.0 0.0000 0.4011667  0.9035514
#> ENSMUSG00000000049.11   0 0.3000000    0.3 0.3410 0.0590000 -2.2967628
#> ENSMUSG00000000056.7    0 0.3333333    0.0 0.0000 0.0000000 11.2293023
#>                            UMAP2  state
#> ENSMUSG00000000001.4  -9.4548692   EnhG
#> ENSMUSG00000000028.15 -0.3666459   EnhG
#> ENSMUSG00000000031.16  9.5740863 EnhBiv
#> ENSMUSG00000000037.16  3.6425823  Quies
#> ENSMUSG00000000049.11  8.4852121 ReprPC
#> ENSMUSG00000000056.7  -1.6012913   EnhG
```

It’s an exemple of UMAP representation to visualized the predominent chromatin state in each gene.



Session Information

Here is the output of `sessionInfo()` on the system on which this document was compiled:

```
#> R version 4.1.3 (2022-03-10)
#> Platform: x86_64-conda-linux-gnu (64-bit)
#> Running under: Ubuntu 18.04.6 LTS
#>
#> Matrix products: default
#> BLAS/LAPACK: /home/mcoulee/anaconda3/envs/R_package_3/lib/libopenblasp-r0.3.20.so
#>
```

```
#> locale:
#>  [1] LC_CTYPE=fr_FR.UTF-8       LC_NUMERIC=C
#>  [3] LC_TIME=fr_FR.UTF-8        LC_COLLATE=fr_FR.UTF-8
#>  [5] LC_MONETARY=fr_FR.UTF-8    LC_MESSAGES=fr_FR.UTF-8
#>  [7] LC_PAPER=fr_FR.UTF-8       LC_NAME=C
#>  [9] LC_ADDRESS=C               LC_TELEPHONE=C
#> [11] LC_MEASUREMENT=fr_FR.UTF-8 LC_IDENTIFICATION=C
#>
#> attached base packages:
#> [1] stats     graphics  grDevices utils     datasets  methods   base
#>
#> other attached packages:
#> [1] ChromENVEE_1.1.7
#>
#> loaded via a namespace (and not attached):
#>  [1] Rcpp_1.0.9             lattice_0.20-45        png_0.1-7
#>  [4] prettyunits_1.1.1      ps_1.7.1               assertthat_0.2.1
#>  [7] rprojroot_2.0.3        digest_0.6.29          utf8_1.2.2
#> [10] RSpectra_0.16-1        R6_2.5.1               GenomeInfoDb_1.30.1
#> [13] stats4_4.1.3           evaluate_0.15          highr_0.9
#> [16] ggplot2_3.3.6          pillar_1.8.1           zlibbioc_1.40.0
#> [19] rlang_1.0.5            callr_3.7.1            S4Vectors_0.32.4
#> [22] Matrix_1.4-1           reticulate_1.26        rmarkdown_2.14
#> [25] labeling_0.4.2         splines_4.1.3          devtools_2.4.3
#> [28] stringr_1.4.1          RCurl_1.98-1.8         munsell_0.5.0
#> [31] umap_0.2.9.0           compiler_4.1.3         xfun_0.31
#> [34] askpass_1.1            pkgconfig_2.0.3        BiocGenerics_0.40.0
#> [37] pkgbuild_1.3.1         mgcv_1.8-40            htmltools_0.5.3
#> [40] openssl_2.0.3          tidyselect_1.1.2       tibble_3.1.8
#> [43] GenomeInfoDbData_1.2.7 IRanges_2.28.0         fansi_1.0.3
#> [46] crayon_1.5.1           dplyr_1.0.9            withr_2.5.0
#> [49] bitops_1.0-7           grid_4.1.3             nlme_3.1-158
#> [52] jsonlite_1.8.0         gtable_0.3.1           lifecycle_1.0.2
#> [55] DBI_1.1.3              magrittr_2.0.3         scales_1.2.1
#> [58] cli_3.4.0              stringi_1.7.8          cachem_1.0.6
#> [61] farver_2.1.1           XVector_0.34.0         fs_1.5.2
#> [64] remotes_2.4.2          ellipsis_0.3.2         vctrs_0.4.1
#> [67] generics_0.1.3         tools_4.1.3            glue_1.6.2
#> [70] purrr_0.3.4            processx_3.7.0         pkgload_1.3.0
#> [73] parallel_4.1.3         fastmap_1.1.0          yaml_2.3.5
#> [76] colorspace_2.0-3       GenomicRanges_1.46.1   sessioninfo_1.2.2
#> [79] memoise_2.0.1          knitr_1.39             usethis_2.1.6
```

References

Ernst J, Kellis M. ChromHMM: automating chromatin-state discovery and characterization. Nature Methods, 9:215-216, 2012

Papier scientifique associé

McInnes, Leland, and John Healy. “UMAP: Uniform Manifold Approximation and Projection for Dimension Reduction.” arXiv:1802.03426.

Lawrence M, Huber W, Pagès H, Aboyoun P, Carlson M, Gentleman R, Morgan M, Carey V (2013). “Software for Computing and Annotating Genomic Ranges." PLoS Computational Biology, 9. doi: 10.1371/journal.pcbi.1003118, http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1003118.