ChromENVEE: Chromatin ENVironment and Expression at Enhancer

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Contents

Abstract **1**

Citation **1**

Introduction **1**

Initialization of data **2**

Distribution of chromatin state in the genome **3**

Annotation of enhancer **4**
Annotated enhancer binding to enhancer position 5
Associated gene expression to enhancer 5
Visualization of enhancer annotation 6

Enhancer annotation comparison **8**

Chromatin state gene environment **12**
Coverage of chromatin state in environment binding to TSS regions 12
Predominant state in environment binding to TSS regions 13

Session Information **15**

References **16**

Abstract

Citation

Introduction

ChromENVEE is a package developed to study chromatin state without Hi-C data.

This package implements functions to associate genes with enhancers, to define the chromatin environment of 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 to enhancers and also estimate the chromatin environment of genes.

This package is available for R version >= 3.6.

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

Initialization of data

`colorTable` is a dataframe which contains informations about chromatin state number (`stateNumber`), chromatin state name (`stateName`) and chromatin state color (`colorValue`). This table is necessary for plot generation. `colorValue` accepted as value hex code and/or color name code.

```
data(colorTable)
```

	stateNumber	stateName	colorValue
1	U1	TSSA	#B71C1C
2	U2	TSSFlnk	#E65100
3	U3	TSSFlnkD	#E65100
4	U4	Tx	#43A047
5	U5	TxWk	#1B5E20
6	U6	EnhG	#99FF66
7	U7	EnhG	#99FF66
8	U8	EnhA	#F5B041
9	U9	EnhWk	#FFEB3B
10	U10	ZNFRpts	#48C9B0
11	U11	Het	#B39DDB
12	U12	TssBiv	#880E4F
13	U13	EnhBiv	#666633
14	U14	ReprPC	#424949
15	U15	ReprPCWk	#7B7D7D
16	U16	Quies	#D0D3D4
17	U17	Quies	#D0D3D4
18	U18	Quies	#D0D3D4

`genomeFile` is a dataframe which contains informations about mouse reference genome.

It is generated from an annotation bed file, in the case of this study we used the Ensembl annotation. `genomeFile` should contain information such as chromosome (chr), gene position (start and end), strand information (strand) and gene name (gene_ENS). Score information is suggested but not mandatory.

```
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 dataframe which contains informations about chromatin state.

It is generated with the output of the ChromHMM tool. `chromatinState` should contain information such as chromosome (chr), genomic regions (start and end), chromatin state information (state and state_name) and sample name (name).

```
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
```

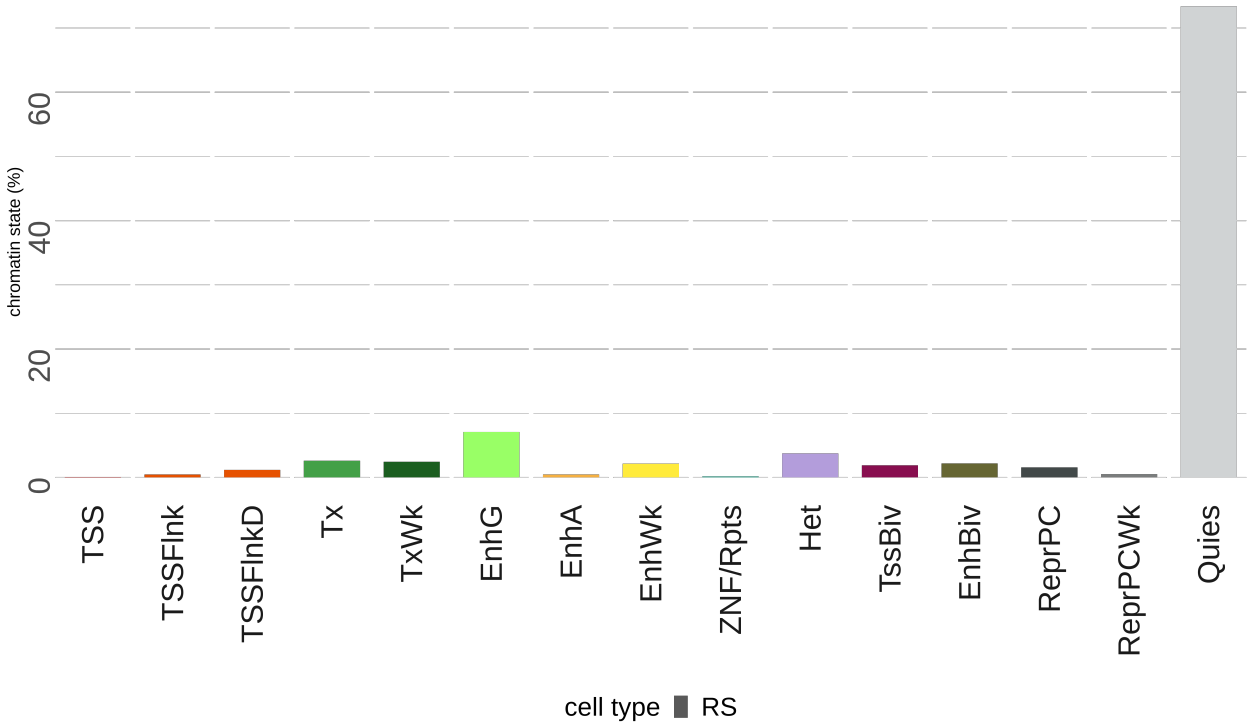
Distribution of chromatin state in the genome

We want to know the distribution of chromatin state in the genome.

`plotChromatinState` calculates the percentage of each chromatin state according to the length of the genome used. We obtain a dataframe 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 dataframe, it's possible to merge all the dataframe in an unique merge dataframe and in an unique plot with the argument `merge = TRUE`.

```
summary_chromatin_state = plotChromatinState(chromatinState, merge = TRUE, plot = FALSE,
colorTable = colorTable, 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 want to associate at each enhancer, all genes regulated by the enhancer. We focus on enhancer chromatin state (in this study, we have 4 types 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 dataframe, `listTableEnhancer` should contain gene and chromatin state informations. Sample name (sample_name) is mandatory if you want to compare enhancer annotation (see Enhancer annotation comparison).

```
data(listTableEnhancer)
```

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

Annotated enhancer binding to enhancer position

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

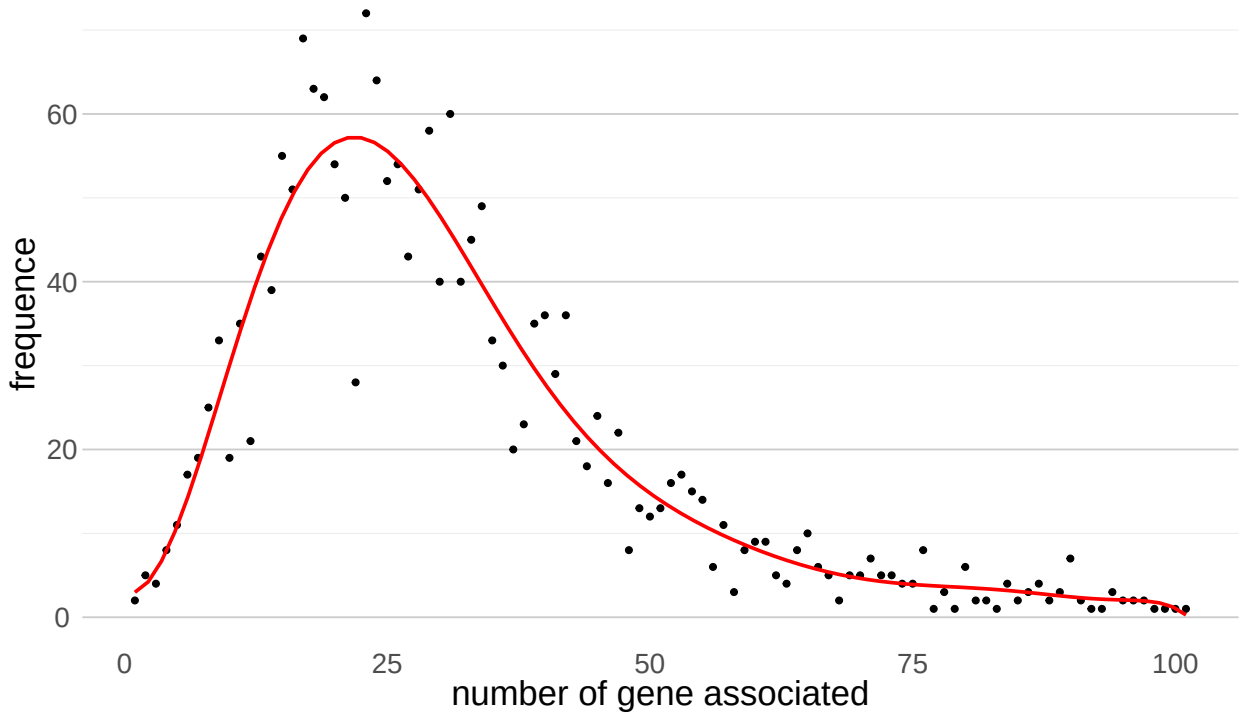
The function takes few minutes to process depending on the length of your enhancer table. It's possible to multithread the function with the `nCore` parameter. For each enhancer position, we get two informations, distance between gene and enhancer (in bp) and gene associate.

```
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 using `plotGeneAssociation`.

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



Associated gene expression to enhancer

`geneExpression` is a dataframe which contains gene expression level information.

It is generated with RNAseq gene expression analysis. `geneExpression` should contain 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 necessary for the analysis. For gene name, you must use the same gene name you used to generate `genomeFile` dataframe because we use the annotation to associate the gene-enhancer pair 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 want to associate the level of gene expression at each gene-enhancer pair are estimated with `enhancerAnnotation` function.

According to `geneExpression` dataframe, it's possible that gene-enhancer pair has not expression level, in this case, function return NA value.

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

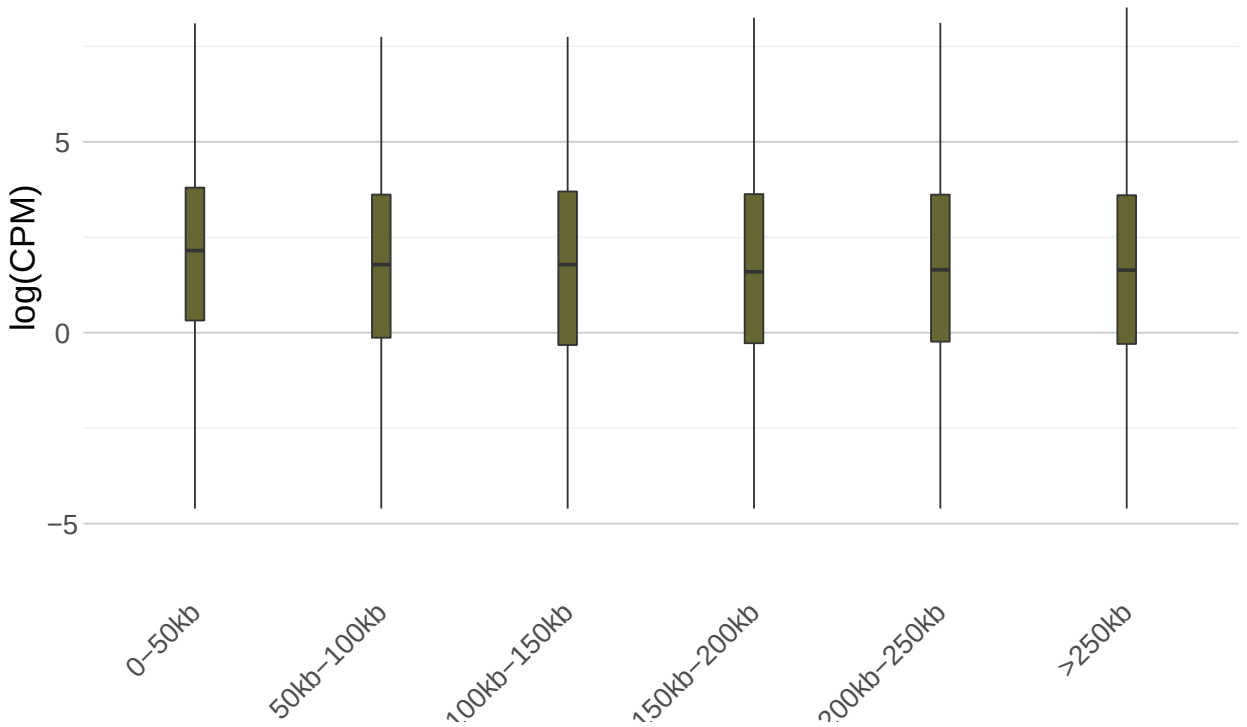
```
#> GRanges object with 6 ranges and 8 metadata columns:
#>     seqnames            ranges strand | chromatin_state sample_name start_500kb
#>        <Rle>         <IRanges>  <Rle> |     <character> <character>   <numeric>
#>   1    chr10   9164400-9164800      * |             U13      EnhBiv    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

Visualization of enhancer annotation

Expression of gene associated to enhancer according to their distance

We generated plot to estimate the level of gene expression according to the distance between gene and enhancer using `plotDistanceExpression`. The distance is estimated with `limit` argument and clusterized in 6 distance groups like the following plot.

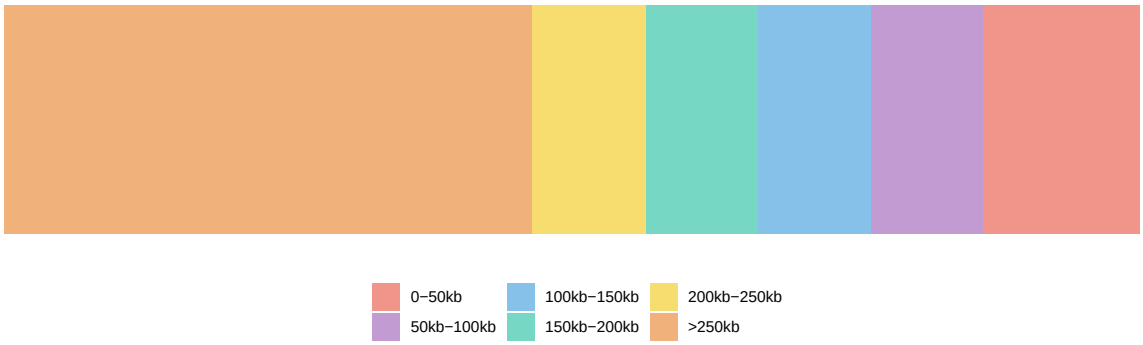
```
plotDistanceExpression(table_enhancer_gene_expression, colorTable = colorTable,
limit = 500000)
```



Distribution of gene according to distance between gene and enhancer

We generated plot to estimate the distribution of gene according to the distance between gene and enhancer using `plotGeneDistance`. The distance is estimated with `limit` argument and clusterized in 6 distance groups like the following plot.

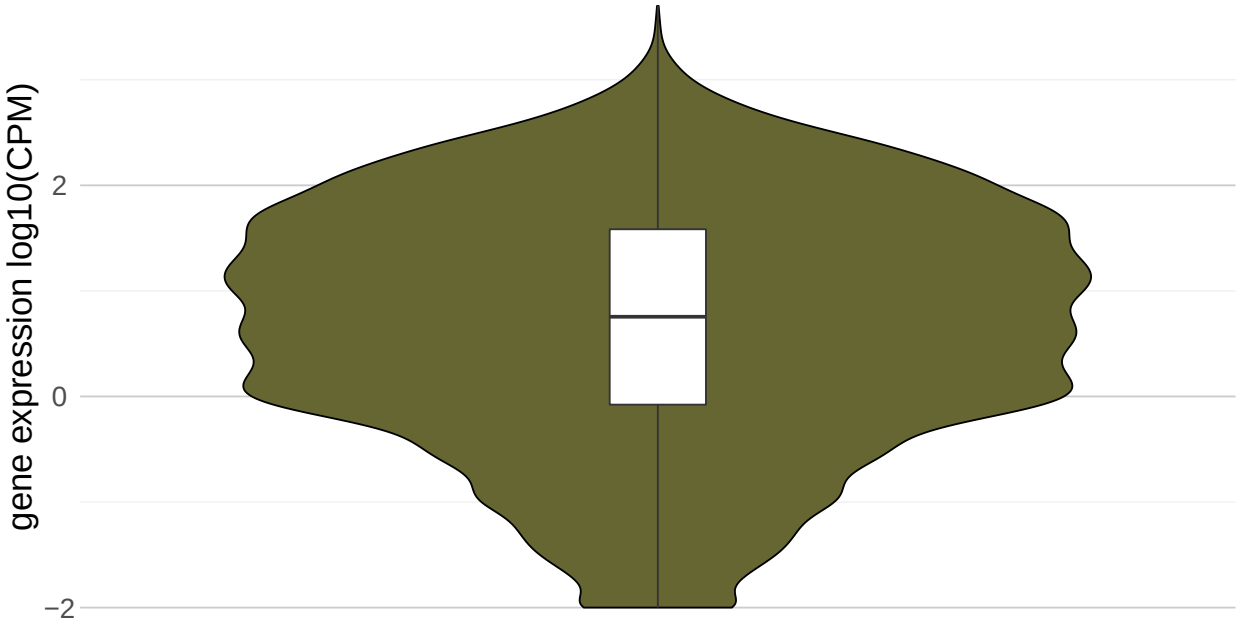
```
plotGeneDistance(table_enhancer_gene_expression, limit = 500000, xlab = "",
ylab = "distance enhancer-gene (bp)")
```



Expression of gene associated to enhancer

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

```
plotEnhancerExpression(table_enhancer_gene_expression, scale = "log10",
colorTable = colorTable, ylab = "gene expression log10(CPM)")
```



Enhancer annotation comparison

It's possible to compare different categories of enhancers. To do this, it's necessary to use a list of GRanges objects, each containing data like those in `listTableEnhancer`. Unlike the individual analysis, each GRanges object in the list requires sample information (sample_name).

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

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

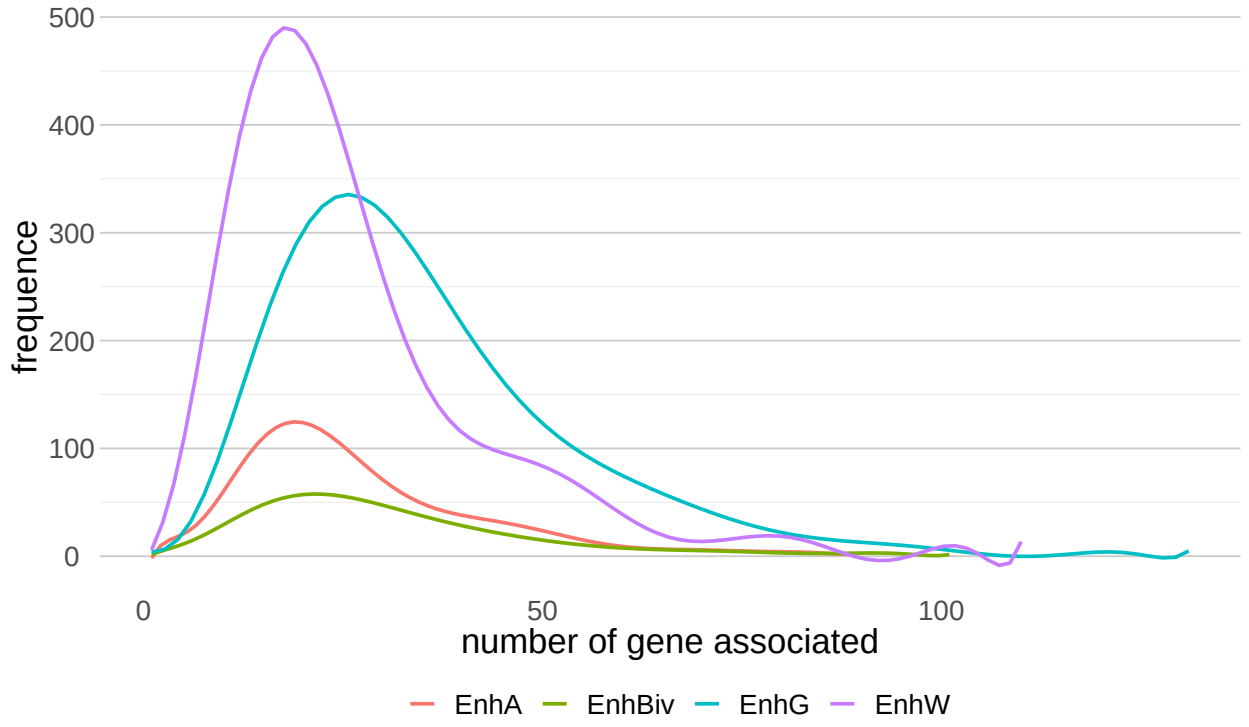
This process take many time. To reduce time, you can load the `listTableEnhancerGeneExpression` data to process the following analyses.

```
data(listTableEnhancerGeneExpression)
```

Number of gene associate at the enhancer

We want to know the distribution of genes associated at each enhancer using `plotGeneAssociation`. `all = TRUE` parameter is used to compile all enhancer tables in same '.png' file.

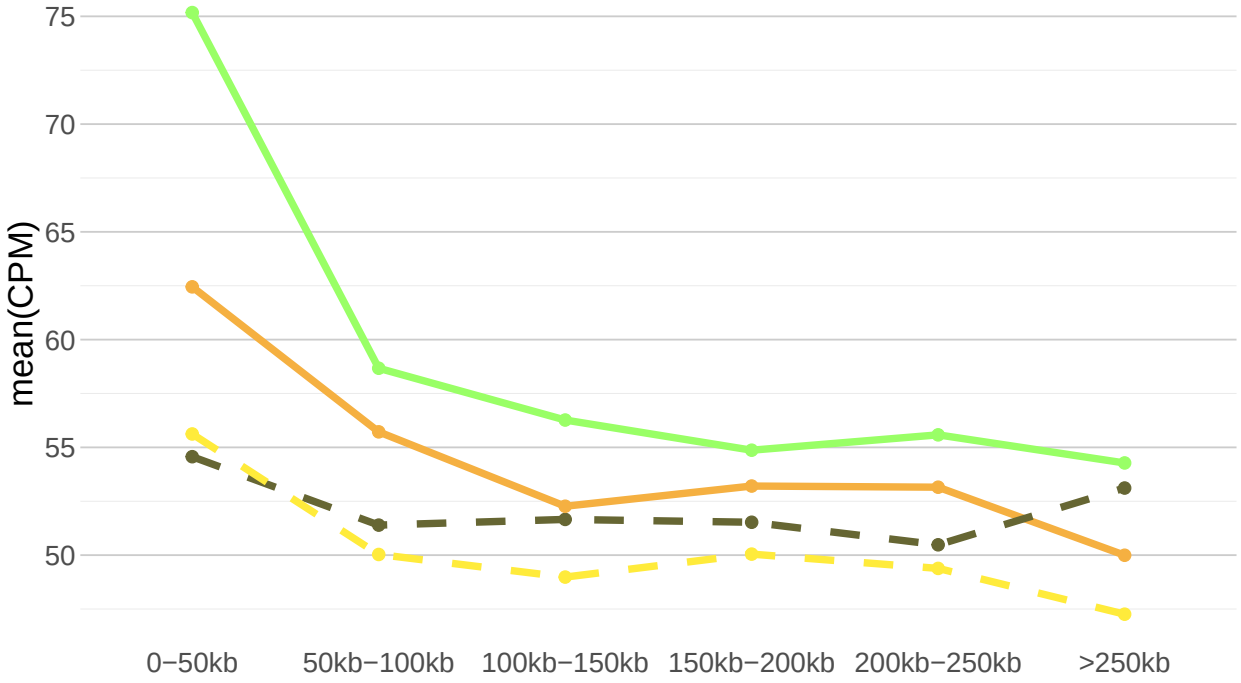
```
plotGeneAssociation(listTableEnhancerGeneExpression, all = TRUE)
```



Expression of gene associated to enhancer according to their distance

We generated plot to estimate the level of gene expression according to the distance between gene and enhancer using `plotDistanceExpression`. The distance is estimated with `limit` argument and clusterized in 6 distance groups like the following plot. In the case of list analysis, the function showed the average of the expression associate to each enhancer.

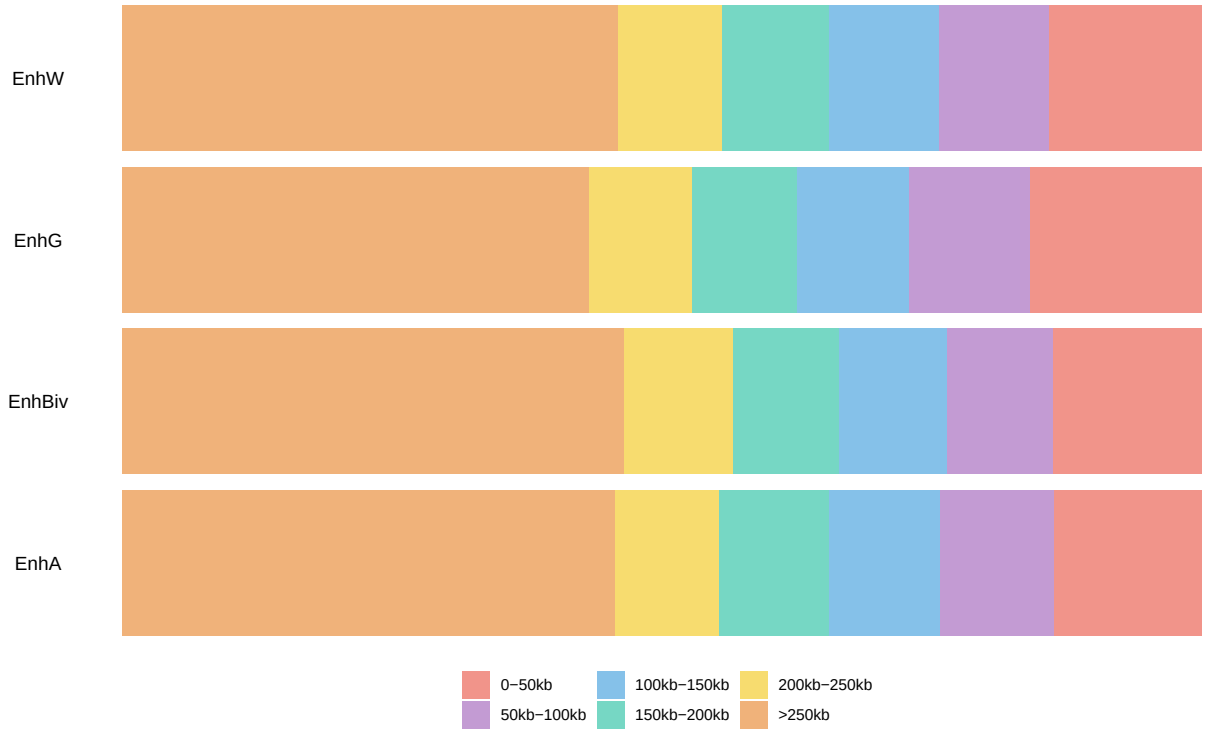
```
plotDistanceExpression(listTableEnhancerGeneExpression, colorTable = colorTable,
limit = 500000)
```



Distribution of gene according to distance between gene and enhancer

We generate plot to estimated the distribution of gene according to the distance between gene and enhancer using `plotGeneDistance`. The distance is estimated with `limit` argument and clusterized in 6 distance groups like the following plot.

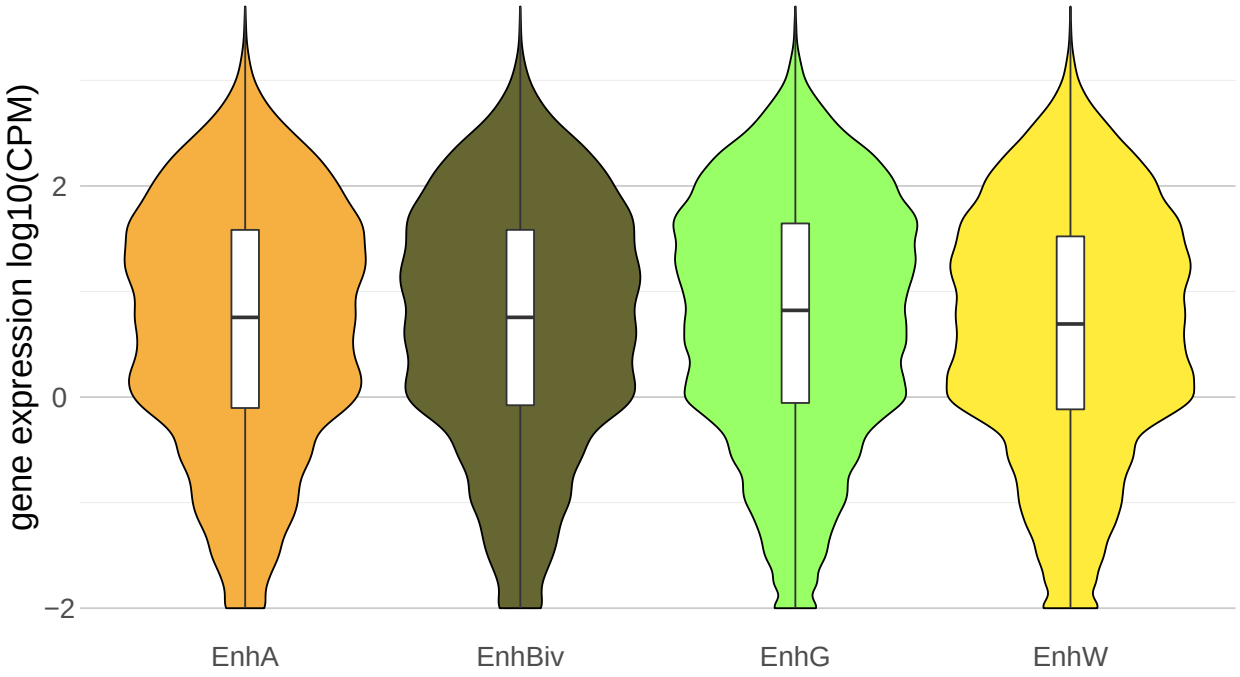
```
plotGeneDistance(listTableEnhancerGeneExpression, limit = 500000,
xlab = "", ylab = "distance enhancer-gene (bp)")
```



Expression of gene associated to enhancer

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

```
plotEnhancerExpression(listTableEnhancerGeneExpression, scale = "log10",
colorTable = colorTable, ylab = "gene expression log10(CPM)")
```



Chromatin state gene environment

We want to study the chromatin environment of gene. To do this, we need gene information data (`geneExpression`) and chromatin state data (`chromatinState`).

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

Coverage of chromatin state in environment binding to TSS regions

`geneEnvironment()` is a function able to estimate the chromatin state environment of gene. For this, we estimated the size of the environment around gene TSS with `interval` parameter. For each gene, we obtain information about the coverage of each chromatin state (`state` parameter) in the environment.

`geneEnvironment()` may take few minutes depending on the number of genes analyzed.

```
table_overlapping = geneEnvironment(geneExpression, chromatinState,
stateOrder = unique(colorTable$stateName), interval = 3000)
rownames(table_overlapping) = table_overlapping$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 TSSFlnkD Tx TxWk
#> ENSMUSG00000000001.4     108149146 0.00000000 0.00000000        0  0    0
#> ENSMUSG00000000028.15     18814987 0.00000000 0.06666667        0  0    0
#> ENSMUSG00000000031.16    142581143 0.00000000 0.00000000        0  0    0
#> ENSMUSG00000000037.16    161120193 0.03333333 0.40000000        0  0    0
#> ENSMUSG00000000049.11    108346354 0.00000000 0.00000000        0  0    0
#> ENSMUSG00000000056.7     121240253 0.00000000 0.06666667        0  0    0
#>                            EnhG EnhA  EnhWk ZNFRpts Het    TssBiv EnhBiv ReprPC
#> ENSMUSG00000000001.4  0.7423333    0 0.0000       0   0 0.2576667    0.0 0.0000
#> ENSMUSG00000000028.15 0.6333333    0 0.0000       0   0 0.3000000    0.0 0.0000
#> ENSMUSG00000000031.16 0.0000000    0 0.0000       0   0 0.0000000    0.4 0.3095
#> ENSMUSG00000000037.16 0.0000000    0 0.1655       0   0 0.0000000    0.0 0.0000
#> ENSMUSG00000000049.11 0.0000000    0 0.0000       0   0 0.3000000    0.3 0.3410
#> ENSMUSG00000000056.7  0.6000000    0 0.0000       0   0 0.3333333    0.0 0.0000
#>                       ReprPCWk     Quies
#> ENSMUSG00000000001.4         0 0.0000000
#> ENSMUSG00000000028.15        0 0.0000000
#> ENSMUSG00000000031.16        0 0.2905000
#> ENSMUSG00000000037.16        0 0.4011667
#> ENSMUSG00000000049.11        0 0.0590000
#> ENSMUSG00000000056.7         0 0.0000000
```

Predominant state in environment binding to TSS regions

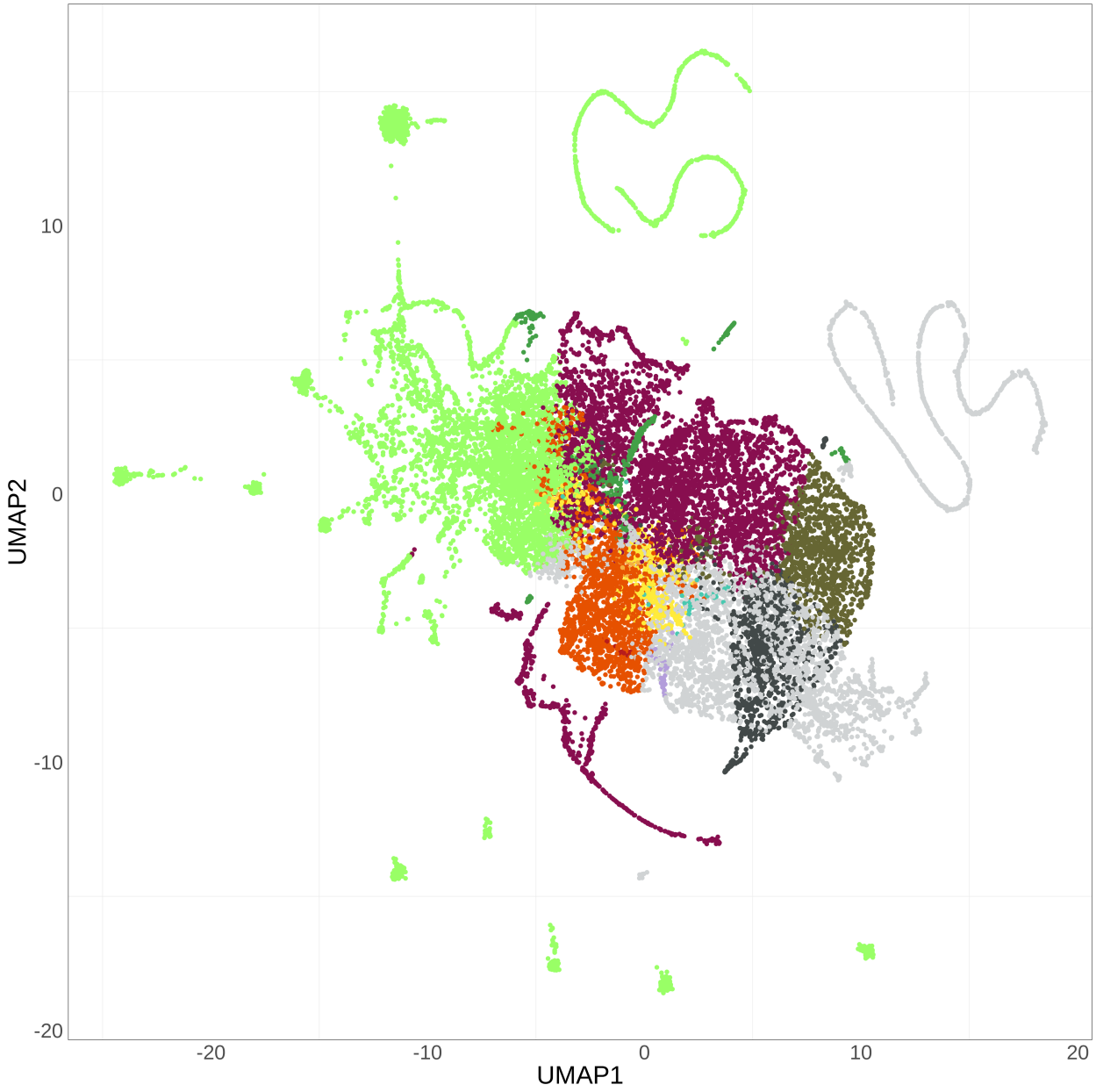
`predominantState()` estimates the predominant chromatin state in the gene environment. The function estimates as predominant the chromatin state with the highest coverage in the environment. Genes are clusterized according to their chromatin state using `umap` package. The function returns a dataframe with information about the predominant chromatin state and UMAP dimension.

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

```
#>                            TSSA    TSSFlnk TSSFlnkD Tx TxWk      EnhG EnhA
#> ENSMUSG00000000001.4  0.00000000 0.00000000        0  0    0 0.7423333    0
#> ENSMUSG00000000028.15 0.00000000 0.06666667        0  0    0 0.6333333    0
#> ENSMUSG00000000031.16 0.00000000 0.00000000        0  0    0 0.0000000    0
#> ENSMUSG00000000037.16 0.03333333 0.40000000        0  0    0 0.0000000    0
#> ENSMUSG00000000049.11 0.00000000 0.00000000        0  0    0 0.0000000    0
#> ENSMUSG00000000056.7  0.00000000 0.06666667        0  0    0 0.6000000    0
#>                        EnhWk ZNFRpts Het    TssBiv EnhBiv ReprPC ReprPCWk
#> ENSMUSG00000000001.4  0.0000       0   0 0.2576667    0.0 0.0000        0
#> ENSMUSG00000000028.15 0.0000       0   0 0.3000000    0.0 0.0000        0
#> ENSMUSG00000000031.16 0.0000       0   0 0.0000000    0.4 0.3095        0
#> ENSMUSG00000000037.16 0.1655       0   0 0.0000000    0.0 0.0000        0
#> ENSMUSG00000000049.11 0.0000       0   0 0.3000000    0.3 0.3410        0
#> ENSMUSG00000000056.7  0.0000       0   0 0.3333333    0.0 0.0000        0
```

```
#>                          Quies       UMAP1      UMAP2  state
#> ENSMUSG00000000001.4  0.0000000 -15.3955897 -12.093308   EnhG
#> ENSMUSG00000000028.15 0.0000000   3.1386058  11.433336   EnhG
#> ENSMUSG00000000031.16 0.2905000  -0.1952710  -8.802595 EnhBiv
#> ENSMUSG00000000037.16 0.4011667  -4.7806753  -2.087036  Quies
#> ENSMUSG00000000049.11 0.0590000  -0.9630213  -5.769000 ReprPC
#> ENSMUSG00000000056.7  0.0000000   2.4736888  11.123211   EnhG
```

It's an exemple of UMAP representation to visualize the predominant chromatin state in each gene. Each point corresponds to a gene and each is colored according to its predominant chromatin state



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] grid      stats     graphics  grDevices utils     datasets  methods
#> [8] base
#>
#> other attached packages:
#> [1] gridExtra_2.3    ChromENVEE_1.1.8
#>
#> 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           nlme_3.1-158           jsonlite_1.8.0
#> [52] gtable_0.3.1           lifecycle_1.0.2        DBI_1.1.3
#> [55] magrittr_2.0.3         scales_1.2.1           cli_3.4.0
#> [58] stringi_1.7.8          cachem_1.0.6           farver_2.1.1
#> [61] XVector_0.34.0         fs_1.5.2               remotes_2.4.2
#> [64] ellipsis_0.3.2         vctrs_0.4.1            generics_0.1.3
#> [67] tools_4.1.3            glue_1.6.2             purrr_0.3.4
#> [70] processx_3.7.0         pkgload_1.3.0          parallel_4.1.3
#> [73] fastmap_1.1.0          yaml_2.3.5             colorspace_2.0-3
#> [76] GenomicRanges_1.46.1   sessioninfo_1.2.2      memoise_2.0.1
#> [79] 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.