Getting Started with EpiRomics

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Contents

Abstract

Summary epiRomics is an R package designed to integrate multi-omics data in order to identify and visualize enhancer regions alongside gene expression and other epigenomic modifications. Regulatory network analysis can be done using combinatory approaches to infer regions of significance such as enhancers, when combining ChIP and histone data. Downstream analysis can identify co-occurrence of these regions of interest with

other user-supplied data, such as chromatin availability or gene expression. Finally, this package allows for results to be visualized at high resolution in a stand-alone browser.

Availability and Implementation epiRomics is released under Artistic-2.0 License. The source code and documents are freely available through Github (https://github.com/Huising-Lab/epiRomics).

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Supplementary information Supplementary data, and methods are available online on *biorXiv* or *Github.*

Competing Interest Statement

The authors have declared no competing interest.

Citation

If you use epiRomics in published research, please cite:

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Loading the epiRomics package and dependencies for vignette

```
## loading packages

library(epiRomics)
#> epiRomics package loaded.
#> Automatic dependency check and verification of sample data presence
#> Bioconductor version 3.13 (BiocManager 1.30.16), R 4.1.1 (2021-08-10)
#> Old packages: 'data.table', 'desc', 'hms', 'knitr', 'libcoin', 'mime', 'party',
#>   'pillar', 'R.utils', 'rcmdcheck', 'readr', 'rJava', 'S4Vectors', 'TH.data',
#>   'tidyr', 'tinytex'
#> looking for  data.table
#> looking for  party
#> Loading required namespace: party
#> looking for  plyr
#> Loading required namespace: plyr
#> looking for  knitr
#> looking for  rmarkdown
#> looking for  AnnotationDbi
#> Loading required namespace: AnnotationDbi
#> looking for  annotatr
#> Loading required namespace: annotatr
#> Warning: replacing previous import 'AnnotationHub::hubUrl' by
#> 'rtracklayer::hubUrl' when loading 'annotatr'
#> looking for  BiocGenerics
#> looking for  GenomicFeatures
#> looking for  GenomicRanges
#> looking for  Gviz
#> Loading required namespace: Gviz
#> looking for  IRanges
```

```
#> looking for  rtracklayer
#> looking for  org.Hs.eg.db
#> Loading required namespace: org.Hs.eg.db
#>
#> looking for  TxDb.Hsapiens.UCSC.hg38.knownGene
#> Loading required namespace: TxDb.Hsapiens.UCSC.hg38.knownGene
#> You are ready to go. For feedback, please email: ammawla@ucdavis.edu
library(TxDb.Hsapiens.UCSC.hg38.knownGene)
#> Loading required package: GenomicFeatures
#> 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.max, which.min
#> Loading required package: S4Vectors
#> Loading required package: stats4
#>
#> Attaching package: 'S4Vectors'
#> The following objects are masked from 'package:base':
#>
#>     expand.grid, I, unname
#> Loading required package: IRanges
#> Loading required package: GenomeInfoDb
#> Loading required package: GenomicRanges
#> Loading required package: AnnotationDbi
#> 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")'.
library(org.Hs.eg.db)
```

Brief explanation of example data

This package includes some example data to get you started, delineating human pancreatic islet enhancers between alpha and beta cells.

Human pancreatic islet alpha and beta ATAC- and companion RNA- Seq data were retrieved from GEO accession GSE76268 (Ackermann, et al., 2016).

ATAC samples were processed using the ENCODE-DCC ATAC sequencing pipeline, aligning to the hg38 (Harrow, et al., 2012) build of the human genome (Consortium, 2012; Davis, et al., 2018).

Peak calls generated through the pipeline using MACS2 (Zhang, et al., 2008) were analyzed downstream through the BioConductor package DiffBind (Ross-Innes, et al., 2012) in order to identify differentially enriched chromatin regions between the two cell types.

RNA samples were quality controlled using the tool fastp (Chen, et al., 2018), and aligned using STAR (Dobin, et al., 2013) to the hg38 build of the human genome. Wiggle files produced by the STAR aligner were then merged by cell type using UCSC command line tools.

Bigwigs merged by cell type were subsetted to chromosome 1 using UCSC command line tools (Kent, et al., 2010).

ChIP-sequencing peak calls generated using MACS2 for human pancreatic islet transcription factors Foxa2, MafB, Nkx2.2, Nkx6.1, and Pdx1 were retrieved from the EMBL-EBI repository database E-MTAB-1919 (Pasquali, et al., 2014). All peak calls were lifted over to the hg38 genome build using the UCSC genome browser liftOver tool (Kent, et al., 2002).

Histone-sequencing peak calls generated using MACS2 for histones H3k27ac and H3k4me1 were retrieved from GEO accession GSE16256 (Bernstein, et al., 2010), and for histone H2A.Z from the EMBL-EBI repository database E-MTAB-1919 (Pasquali, et al., 2014). All peak calls were lifted over to the hg38 genome build using the UCSC genome browser liftOver tool.

The FANTOM5 human enhancer database (Lizio, et al., 2015) was retrieved, and all regions were lifted over to the hg38 genome build using the UCSC genome browser liftOver tool.

Human ultra-conserved non-coding elements (UCNEs) were retrieved form the UCNE database (Dimitrieva and Bucher, 2012), and all regions were lifted over to the hg38 genome build using the UCSC genome browser liftOver tool.

The human islet regulome database was retrieved (Miguel-Escalada, et al., 2019) and all regions were lifted over to the hg38 genome build using the UCSC genome browser liftOver tool.

Lets load and take a look at how to properly format the datasets epiRomics uses to build the initial database.

```
## Required columns are: name, path, genome, format, and type

## The genome must also be in proper format, e.g. mm10 or hg38

## Type of data can be histone, methyl, SNP, or ChIP. ChIP is
## required for some downstream functions to work appropriately.

example_epiRomics_Db_sheet <- read.csv(file = system.file("extdata", "example_epiRomics_Db_sheet_user_pa
    package = "epiRomics"))

head(example_epiRomics_Db_sheet)
```

name	path	genome	format	type
h3k27ac	/Library/Frameworks/R.framework/Versions/4.1/Resources/library/epiRomics/extdata/Histone/H3k27ac_hg38.be	hg38	bed	Histone
h3k4me1	/Library/Frameworks/R.framework/Versions/4.1/Resources/library/epiRomics/extdata/Histone/H3K4me1_hg38.b	hg38	bed	Histone
foxa2	/Library/Frameworks/R.framework/Versions/4.1/Resources/library/epiRomics/extdata/ChIP/FOXA2_hg38.bed	hg38	bed	ChIP
mafb	/Library/Frameworks/R.framework/Versions/4.1/Resources/library/epiRomics/extdata/ChIP/MAFB_hg38.bed	hg38	bed	ChIP
nkx2_2	/Library/Frameworks/R.framework/Versions/4.1/Resources/library/epiRomics/extdata/ChIP/NKX2_2_hg38.bed	hg38	bed	ChIP

name	path	genome	format	type
nxk6_1	/Library/Frameworks/R.framework/Versions/4.1/Resources/library/epiRomics/extdata/ChIP/NKX6_1_hg38.bed	hg38	bed	ChIP

How to load and build the database

```
## epiRomics_build_dB constructs a database of class epiRomics with this data sheet

epiRomics_dB <- epiRomics_build_dB(epiRomics_db_file = system.file("extdata", "example_epiRomics_Db_she
    package = "epiRomics"), txdb_organism = "TxDb.Hsapiens.UCSC.hg38.knownGene::TxDb.Hsapiens.UCSC.hg38
    epiRomics_genome = "hg38", epiRomics_organism = "org.Hs.eg.db")
#>
#> Attaching package: 'Biostrings'
#> The following object is masked from 'package:base':
#>
#>     strsplit
#> Building enhancers...
#> snapshotDate(): 2021-05-18
#> loading from cache
#> 'select()' returned 1:1 mapping between keys and columns
#> Building promoters...
#> Building 1to5kb upstream of TSS...
#> Building intergenic...
#> Building cds...
#> Building 5UTRs...
#> Building 3UTRs...
#> Building exons...
#> Building first exons...
#> Building introns...
#> Building intron exon boundaries...
#> Building exon intron boundaries...
#> Building CpG islands...
#> Building CpG shores...
#> Building CpG shelves...
#> Building inter-CpG-islands...
#> snapshotDate(): 2021-05-18
#> Building lncRNA transcripts...
#> loading from cache
```

Delineating active enhancers using H3k4me1 and H3k27ac marks as a proxy

```
## Identifying active, putative enhancers

# 3 There is a lot of flexibility for data exploration here. In this
# example, we search for putative enhancers using two histone marks
# known to co-occur at enhancer regions - h3k4me1 & h3k27ac
```

```
epiRomics_putative_enhancers <- epiRomics_enhancers(epiRomics_dB, epiRomics_histone_mark_1 = "h3k4me1",
    epiRomics_histone_mark_2 = "h3k27ac")

## Taking a look, we see a list of 19,692 putative enhancers demarked
## by H3k4me1 & H3k27ac

epiRomics_putative_enhancers@annotations
#> GRanges object with 19692 ranges and 0 metadata columns:
#>           seqnames            ranges strand
#>              <Rle>         <IRanges>  <Rle>
#>       [1]     chr1    999886-1000011      *
#>       [2]     chr1   1000228-1000811      *
#>       [3]     chr1   1000850-1001468      *
#>       [4]     chr1   1005007-1006023      *
#>       [5]     chr1   1013701-1013893      *
#>       ...      ...               ...    ...
#>   [19688]     chrY 12392544-12392994      *
#>   [19689]     chrY 13282680-13282760      *
#>   [19690]     chrY 15455449-15455788      *
#>   [19691]     chrY 19066496-19066508      *
#>   [19692]     chrY 19075542-19075899      *
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
```

Cross-referencing enhancer calls to other databases

FANTOM Enhancer Database

```
## Now we have a list of regions as possible candidates for
## enhancers, but where do we go from here? One way to increase
## confidence of these calls is to cross this list against an
## enhancer database, for instance, FANTOM.

## NOTE: This option may not be available for all organisms.

epiRomics_putative_enhancers_filtered_fantom <- epiRomics_enhancers_filter(epiRomics_putative_enhancers
    epiRomics_dB, epiRomics_type = "hg38_custom_fantom")

## Taking a look, we see a reduced number of 2,749 candidate regions

epiRomics_putative_enhancers_filtered_fantom@annotations
#> GRanges object with 2749 ranges and 0 metadata columns:
#>          seqnames              ranges strand
#>             <Rle>           <IRanges>  <Rle>
#>      [1]     chr1     1021242-1021277      *
#>      [2]     chr1     1021318-1021698      *
#>      [3]     chr1     1079632-1080061      *
#>      [4]     chr1     1080101-1080628      *
#>      [5]     chr1     1128200-1128445      *
#>      ...      ...                 ...    ...
```

```
#>   [2745]     chrX 154369950-154370183      *
#>   [2746]     chrX 154371971-154372237      *
#>   [2747]     chrX 154372350-154372695      *
#>   [2748]     chrX 154517139-154517596      *
#>   [2749]     chrX 154734550-154734738      *
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
```

Human Pancreatic Islet Regulome Enhancer Database

```
## We can also filter putative enhancer calls against active
## enhancers from the human islet regulome database

epiRomics_putative_enhancers_filtered_regulome_active <- epiRomics_enhancers_filter(epiRomics_putative_
    epiRomics_dB, epiRomics_type = "hg38_custom_regulome_active")


epiRomics_putative_enhancers_filtered_regulome_active@annotations
#> GRanges object with 6025 ranges and 0 metadata columns:
#>          seqnames              ranges strand
#>             <Rle>           <IRanges>  <Rle>
#>      [1]     chr1     1068896-1068951      *
#>      [2]     chr1     1069171-1069333      *
#>      [3]     chr1     1079632-1080061      *
#>      [4]     chr1     1080101-1080628      *
#>      [5]     chr1     1158358-1158930      *
#>      ...      ...                 ...    ...
#>   [6021]     chrX 153381411-153381523      *
#>   [6022]     chrX 153381677-153381956      *
#>   [6023]     chrX 153382322-153382448      *
#>   [6024]     chrX 153985442-153985689      *
#>   [6025]     chrX 154091801-154091996      *
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
```

Human Pancreatic Islet Regulome Super-Enhancer Database

```
## We can also filter putative enhancer calls against super enhancers
## from human islet regulome database

epiRomics_putative_enhancers_filtered_regulome_super <- epiRomics_enhancers_filter(epiRomics_putative_e
    epiRomics_dB, epiRomics_type = "hg38_custom_regulome_super")

epiRomics_putative_enhancers_filtered_regulome_super@annotations
#> GRanges object with 2401 ranges and 0 metadata columns:
#>          seqnames            ranges strand
#>             <Rle>         <IRanges>  <Rle>
#>      [1]     chr1   7574092-7574479      *
#>      [2]     chr1   7574640-7575094      *
```

```
#>      [3]     chr1   8169274-8169689      *
#>      [4]     chr1   8170112-8170857      *
#>      [5]     chr1   8174089-8174358      *
#>      ...      ...               ...    ...
#>   [2397]    chr22 46109916-46110442      *
#>   [2398]    chr22 46115774-46116154      *
#>   [2399]    chr22 46116326-46116501      *
#>   [2400]     chrX 39813348-39813627      *
#>   [2401]     chrX 39814304-39814607      *
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
```

Human Ultra-Conserved Non-Coding Elements Database

```
## We can also filter putative enhancer calls against Ultra-Conserved
## Non-Coding Elements

epiRomics_putative_enhancers_filtered_ucnes <- epiRomics_enhancers_filter(epiRomics_putative_enhancers,
    epiRomics_dB, epiRomics_type = "hg38_custom_ucnes")


epiRomics_putative_enhancers_filtered_ucnes@annotations
#> GRanges object with 11 ranges and 0 metadata columns:
#>        seqnames              ranges strand
#>           <Rle>           <IRanges>  <Rle>
#>    [1]     chr1 164635220-164635921      *
#>    [2]     chr1 164711914-164712296      *
#>    [3]     chr1 164712350-164713071      *
#>    [4]     chr1 200079185-200079426      *
#>    [5]     chr1 213585694-213586385      *
#>    [6]     chr3   71131859-71132164      *
#>    [7]     chr9 106921420-106921764      *
#>    [8]    chr11 114163425-114164860      *
#>    [9]    chr15   36903894-36904085      *
#>   [10]    chr15   53447393-53447809      *
#>   [11]    chr21   16534340-16534665      *
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
```

Screening for high transcription factor co-binding sites

Biology has established that enhancers can be quite redundant, and not all play an active role in regulating a cell's activity. How can we utilize other epigenomic data in order to identify true enhanceosome regions? One way is to cross this list against all ChIP data of the cell type. A true enhanceosome region should have made it through our filtering thus far, and contain several binding sites for known TFs. Co-binding is expected, and the list is sorted by the highest number of ChIP hits within the region.

```
epiRomics_putative_enhanceosome_fantom <- epiRomics_enhanceosome(epiRomics_putative_enhancers_filtered_
    epiRomics_dB)
```

```
#>
#> >> preparing features information...      2021-09-28 19:40:33
#> >> identifying nearest features...        2021-09-28 19:40:36
#> >> calculating distance from peak to TSS...   2021-09-28 19:40:37
#> >> assigning genomic annotation...        2021-09-28 19:40:37
#> >> adding gene annotation...          2021-09-28 19:42:04
#> 'select()' returned 1:many mapping between keys and columns
#> >> assigning chromosome lengths           2021-09-28 19:42:04
#> >> done...                     2021-09-28 19:42:04
## Taking a look, we see the top candidates meet the criteria we list
## as expected

epiRomics_putative_enhanceosome_fantom@annotations
#> GRanges object with 2749 ranges and 19 metadata columns:
#>        seqnames              ranges strand |     foxa2      mafb    nkx2_2
#>           <Rle>           <IRanges>  <Rle> | <integer> <integer> <integer>
#>    183     chr1 154418514-154419684      * |         2         2         1
#>   1096     chr9     2242369-2242873      * |         2         1         1
#>   2615    chr22   30310745-30311570      * |         2         1         2
#>     34     chr1   10685395-10688670      * |         1         0         1
#>    792     chr6   30748438-30749427      * |         2         1         1
#>    ...      ...                 ...    ... .       ...       ...       ...
#>   2743     chrX 153927339-153927701      * |         0         0         0
#>   2745     chrX 154369950-154370183      * |         0         0         0
#>   2746     chrX 154371971-154372237      * |         0         0         0
#>   2747     chrX 154372350-154372695      * |         0         0         0
#>   2748     chrX 154517139-154517596      * |         0         0         0
#>          nxk6_1      pdx1      h2az ChIP_Hits             annotation   geneChr
#>       <integer> <integer> <integer> <numeric>            <character> <integer>
#>    183         2         1         2        10 Intron (ENST00000622..         1
#>   1096         1         2         1         8     Distal Intergenic         9
#>   2615         1         1         1         8       Promoter (2-3kb)        22
#>     34         1         2         2         7 Intron (ENST00000377..         1
#>    792         1         1         1         7 Intron (ENST00000656..         6
#>    ...       ...       ...       ...       ...                   ...       ...
#>   2743         0         0         0         0       Promoter (<=1kb)        23
#>   2745         0         0         0         0       Promoter (1-2kb)        23
#>   2746         0         0         0         0       Promoter (<=1kb)        23
#>   2747         0         0         0         0       Promoter (1-2kb)        23
#>   2748         0         0         0         0       Promoter (<=1kb)        23
#>        geneStart   geneEnd geneLength geneStrand      geneId      transcriptId
#>        <integer> <integer>  <integer>  <integer> <character>       <character>
#>    183 154429343 154449979      20637          1        3570 ENST00000476006.5
#>   1096   2181571   2186183       4613          1        6595 ENST00000635392.1
#>   2615  30292008  30307890      15883          2       83874 ENST00000403362.5
#>     34  10660737  10693912      33176          2       54897 ENST00000478728.2
#>    792  30743199  30744547       1349          2        8870 ENST00000259874.6
#>    ...       ...       ...        ...        ...         ...                ...
#>   2743 153920715 153926860       6146          2         393 ENST00000422091.1
#>   2745 154348524 154371203      22680          2        2316 ENST00000420627.5
#>   2746 154348529 154371283      22755          2        2316 ENST00000422373.6
#>   2747 154348529 154371283      22755          2        2316 ENST00000422373.6
#>   2748 154506204 154516242      10039          2       60343 ENST00000434658.6
```

```
#>        distanceToTSS         ENSEMBL      SYMBOL                GENENAME
#>            <numeric>     <character> <character>             <character>
#>    183         -9659 ENSG00000160712        IL6R interleukin 6 receptor
#>   1096         60798 ENSG00000080503     SMARCA2 SWI/SNF related, mat..
#>   2615         -2855 ENSG00000099992    TBC1D10A TBC1 domain family m..
#>     34          5242 ENSG00000130940       CASZ1   castor zinc finger 1
#>    792         -3891 ENSG00000137331        IER3 immediate early resp..
#>    ...           ...             ...         ...                    ...
#>   2743          -479 ENSG00000089820     ARHGAP4 Rho GTPase activatin..
#>   2745          1020 ENSG00000196924        FLNA               filamin A
#>   2746          -688 ENSG00000196924        FLNA               filamin A
#>   2747         -1067 ENSG00000196924        FLNA               filamin A
#>   2748          -897 ENSG00000071889       FAM3A FAM3 metabolism regu..
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
## Evaluate calls on chromosome 1
head(as.data.frame(epiRomics_putative_enhanceosome_fantom@annotations)[as.data.frame(epiRomics_putative_
    "chr1", ])
```

	seqnames	start	end	width	strand	fox_a2	fox_a2_afb	fox_a2_xl	fox_a2_xl_6d	fox_h12a2	ChIP_annotation	Histone_gene	gene_strand	Gene_Start	Gene_End	Gene_Length	Gene_Strand	Gene_Id	Transcript_Id	distance	ENSEMBL	SYMBOL	GENENAME
183	chr1	154[illegible]	154[illegible]	1[illegible]	2	2	1	2	1	2	10	Intron (ENST00000622330.4/3570, intron 1 of 6)	1	154[illegible]	154[illegible]	3570	ENST00000622330.4	ENSG00000160712.13	ENST00000622330.4	-9659	ENSG00000160712	IL6R	interleukin 6 receptor
34	chr1	106[illegible]	106[illegible]	1[illegible]	1	0	1	1	2	2	7	Intron (ENST00000377022.8/54897, intron 4 of 20)	1	106[illegible]	106[illegible]	54897	ENST00000377022.8	ENSG00000130940.16	ENST00000377022.8	5242	ENSG00000130940	CASZ1	castor zinc finger 1
24	chr1	81[illegible]	81[illegible]	1[illegible]*	1	1	1	1	1	1	6	Distal Intergenic	1	82[illegible]	82[illegible]	102[illegible]	ENST00000530661.1	ENSG00000227634.6	ENST00000530661.1	30661	ENSG00000227634	NA	intergenic non-protein coding RNA 1714
67	chr1	216[illegible]	216[illegible]	1[illegible]	1	1	1	1	1	1	6	Intron (ENST00000290101.8/5909, intron 2 of 26)	1	215[illegible]	215[illegible]	5909	ENST00000290101.8	ENSG00000117860.14	ENST00000290101.8	10842	ENSG00000117860	ARHGAP25	Rho GTPase activating protein

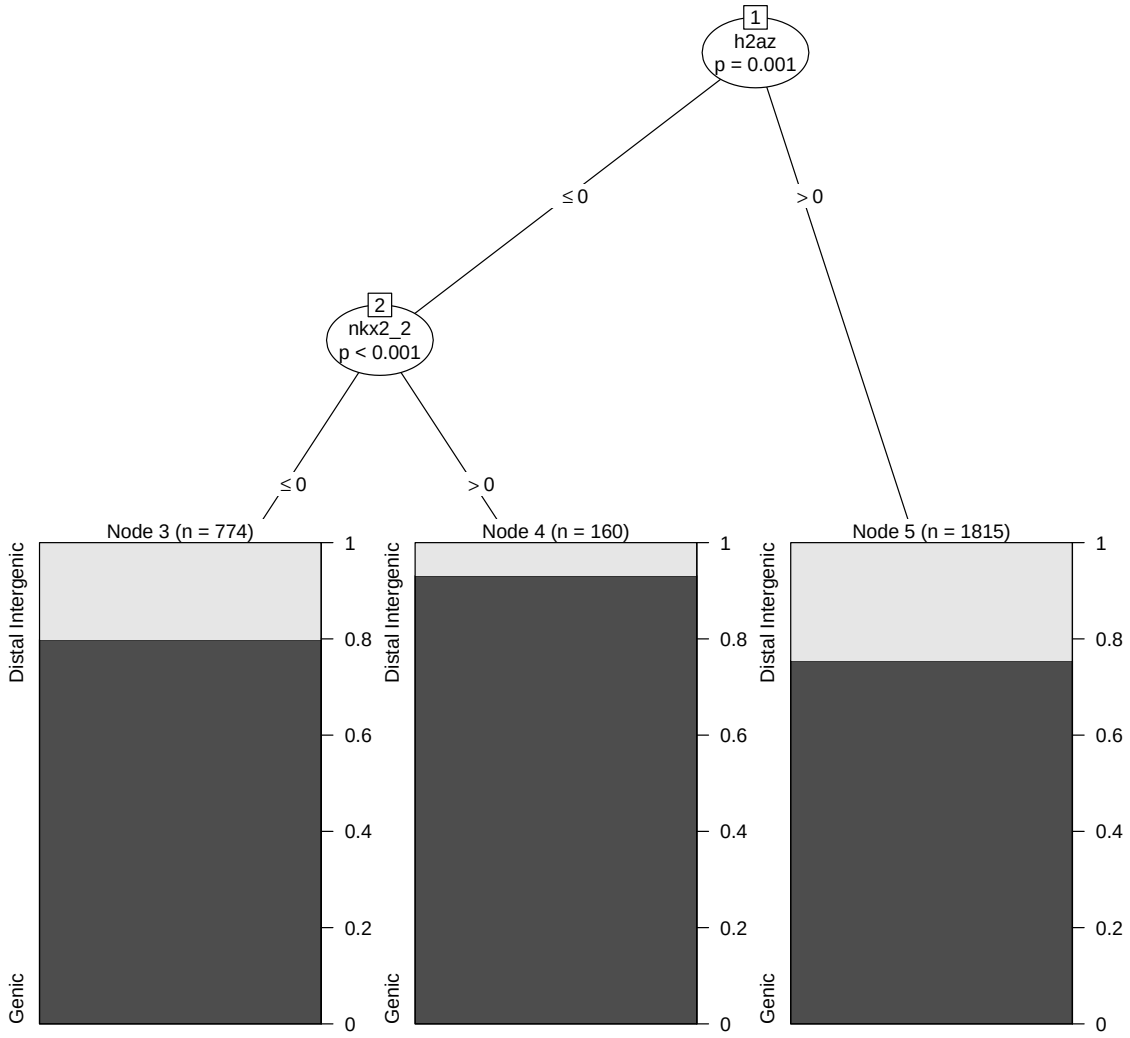
	seqnames	starts	end	width	strand	fold_enrichment	-log10pvalue	-log10qvalue	log2_fold_enrichment	-log10pvalue_sample	-log10qvalue_sample	log2_fold_enrichment_sample	ChIP_annotation	Distance_to_gene	gene_chr	gene_start	gene_end	gene_strand	gene_id	transcript_id	distance_to_TSS	SYMBOL	GENENAME
71	chr1	224[illegible]	[illegible]	1076	1	1	1	1	1	1	1	6	Distal Intergenic	1	224[illegible]	[illegible]	[illegible]	9923	ENST00000414032	ENST00000414032	84677	ZBTB40	zinc finger and BTB domain containing 40
256	chr1	205[illegible]	[illegible]	1660	1	1	2	1	0	1	6	Promoter (2-3kb)	1	205[illegible]	[illegible]	[illegible]	8178	ENST00000367545	ENST00000367545	2465	PTPN14	protein tyrosine kinase 14

```
## Find Index
which(names(epiRomics_putative_enhanceosome_fantom@annotations) == 183)
#> [1] 1
```

Transcription factor decision trees

ChIP dataset repositories are quite sizeable for many organisms and cell types, with the expectation to only grow larger. Many different TFs binding to a putative enhancer region may not be that meaningful in the context of your biological question. A further step would be to ask whether there are co-TFs that pop up together, and whether this pattern varies across the functional annotation of the genome, i.e. does the combination of two TFs on enhanceosomes change on the gene body compared to distal intergenic regions?

```
plot(epiRomics_predictors(epiRomics_putative_enhanceosome_fantom))
```



Intersecting and visualizing ATAC- and RNA-Seq data

What if you wanted to visualize co-binding on your FANTOM filtered putative enhancer region? And do you have additional data you want to include for visualization, such as ATAC and RNA Seq? Lets take a look at one of the top hits

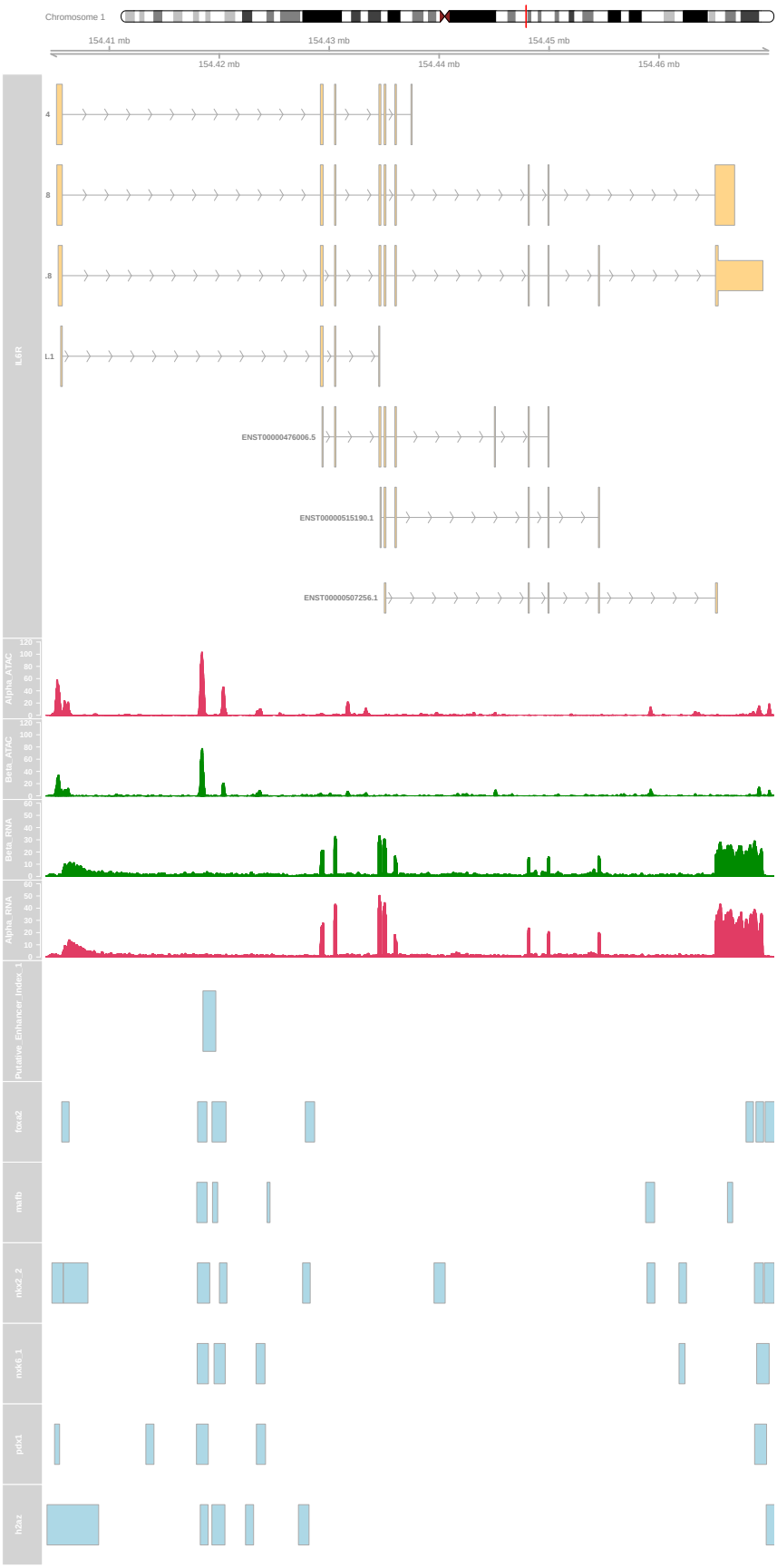
```
## Read in ATAC Seq and RNA Seq track bigwigs

## NOTE: These bigwigs are subsetted to chromosome 1. Indices not
## falling on chromosome 1 will return an error.

epiRomics_track_connection <- read.csv(system.file("extdata", "example_epiRomics_BW_sheet_user_paths.csv
    package = "epiRomics"))
```

```
epiRomics_track_layer_human(
  epiRomics_putative_enhanceosome_fantom,
  epiRomics_index = which(
    names(epiRomics_putative_enhanceosome_fantom@annotations) == 183
  ),
```

```
  epiRomics_dB = epiRomics_dB,
  epiRomics_track_connection = epiRomics_track_connection
)
#> [1] "not empty"
#> [1] 103.678
#> [1] "not empty"
#> [1] 77.5726
#> [1] "not empty"
#> [1] 33.2945
#> [1] "not empty"
#> [1] 50.4959
```


Chromosome 1
154.41 mb
154.42 mb
154.43 mb
154.44 mb
154.45 mb
154.46 mb
IL6R
4
8
.8
L1
ENST00000476006.5
ENST00000515190.1
ENST00000507256.1
Alpha_ATAC
Beta_ATAC
Beta_RNA
Alpha_RNA
Putative_Enhancer_Index_1
foxa2
nsmfb
nkx2_2
nkx6_1
pdx1
h2az

```
## What about a region that overlapped with active enhancers from the
## human islet regulome database?

epiRomics_putative_enhanceosome_regulome_active <- epiRomics_enhanceosome(epiRomics_putative_enhancers_
    epiRomics_dB)
#> >> preparing features information...      2021-09-28 19:46:18
#> >> identifying nearest features...        2021-09-28 19:46:18
#> >> calculating distance from peak to TSS...   2021-09-28 19:46:19
#> >> assigning genomic annotation...        2021-09-28 19:46:19
#> >> adding gene annotation...          2021-09-28 19:46:30
#> 'select()' returned 1:many mapping between keys and columns
#> >> assigning chromosome lengths           2021-09-28 19:46:31
#> >> done...                     2021-09-28 19:46:31
epiRomics_putative_enhanceosome_regulome_active@annotations
#> GRanges object with 6025 ranges and 19 metadata columns:
#>        seqnames              ranges strand |     foxa2      mafb    nkx2_2
#>           <Rle>           <IRanges>  <Rle> | <integer> <integer> <integer>
#>    456     chr1 154418514-154419684      * |         2         2         1
#>   2082     chr7     1555599-1556082      * |         1         1         1
#>   2572     chr9     2242369-2242873      * |         2         1         1
#>   3421    chr11   65416576-65419753      * |         1         1         2
#>   4709    chr17     7887867-7889135      * |         1         0         2
#>    ...      ...                 ...    ... .       ...       ...       ...
#>   5999     chrX            49184194      * |         0         0         0
#>   6001     chrX   70478965-70479351      * |         0         0         0
#>   6006     chrX 107710676-107711066      * |         0         0         0
#>   6007     chrX 107711430-107711673      * |         0         0         0
#>   6020     chrX 150874055-150874330      * |         0         0         0
#>          nxk6_1      pdx1      h2az ChIP_Hits             annotation   geneChr
#>       <integer> <integer> <integer> <numeric>            <character> <integer>
#>    456         2         1         2        10 Intron (ENST00000622..         1
#>   2082         2         2         1         8       Promoter (<=1kb)         7
#>   2572         1         2         1         8      Distal Intergenic         9
#>   3421         1         1         2         8      Distal Intergenic        11
#>   4709         2         2         1         8       Promoter (<=1kb)        17
#>    ...       ...       ...       ...       ...                    ...       ...
#>   5999         0         0         0         0       Promoter (<=1kb)        23
#>   6001         0         0         0         0       Promoter (<=1kb)        23
#>   6006         0         0         0         0      Distal Intergenic        23
#>   6007         0         0         0         0      Distal Intergenic        23
#>   6020         0         0         0         0 Intron (ENST00000370..        23
#>        geneStart   geneEnd geneLength geneStrand      geneId       transcriptId
#>        <integer> <integer>  <integer>  <integer> <character>        <character>
#>    456 154429343 154449979      20637          1        3570  ENST00000476006.5
#>   2082   1550305   1556120       5816          2      202915  ENST00000441933.5
#>   2572   2181571   2186183       4613          1        6595  ENST00000635392.1
#>   3421  65422798  65445540      22743          1      283131  ENST00000501122.2
#>   4709   7888789   7912755      23967          1        1107 ENST00000330494.12
#>    ...       ...       ...        ...        ...         ...                ...
#>   5999  49175621  49184789       9169          2        4007  ENST00000453382.5
#>   6001  70479118  70499903      20786          1        1741  ENST00000466140.5
#>   6006 107714677 107716401       1725          2        1831  ENST00000486554.1
#>   6007 107714677 107716401       1725          2        1831  ENST00000486554.1
```

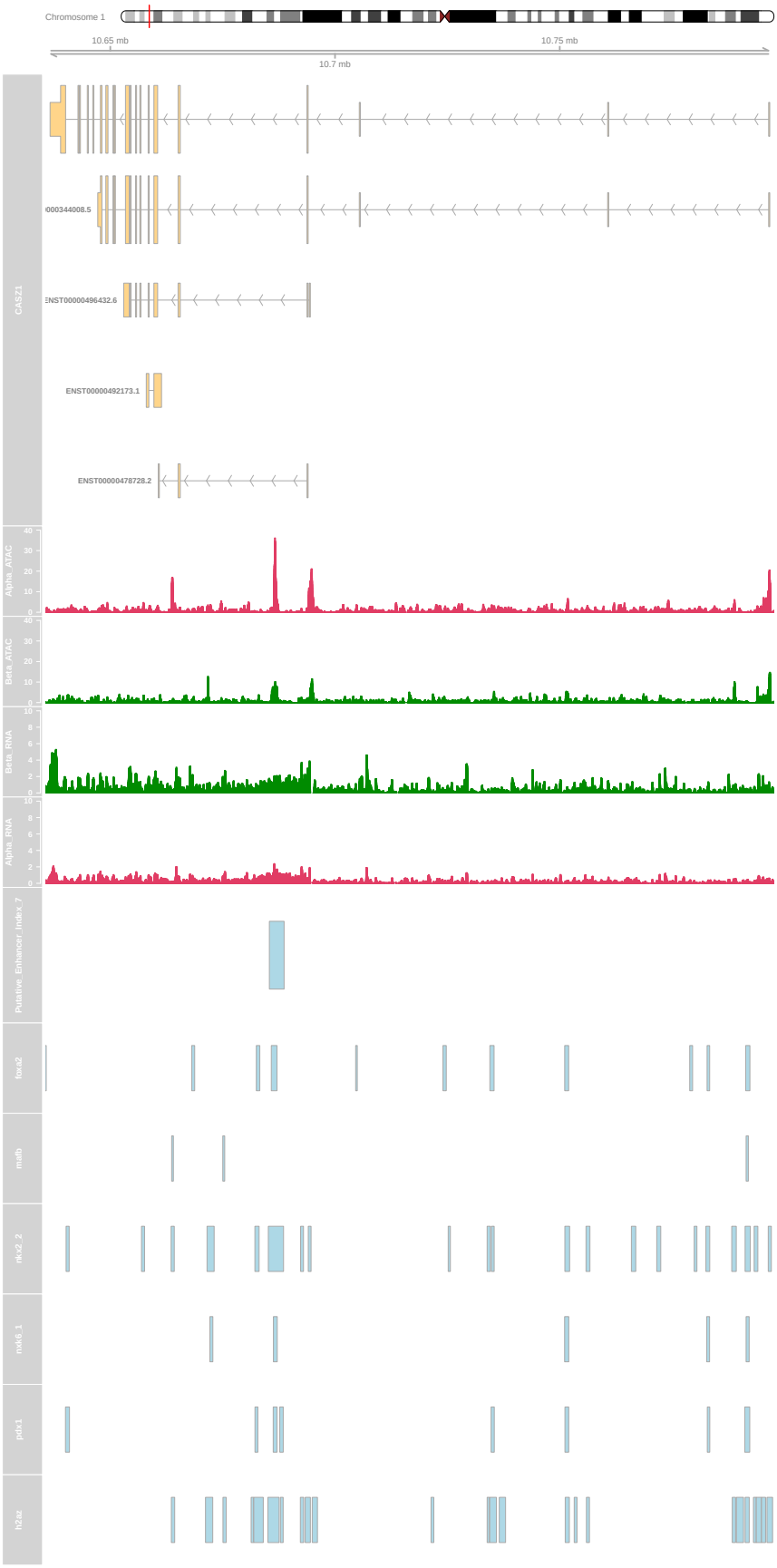
```
#>   6020 150814900 150898609      83710          2       83692  ENST00000491877.1
#>        distanceToTSS         ENSEMBL      SYMBOL                GENENAME
#>            <numeric>     <character> <character>             <character>
#>    456         -9659 ENSG00000160712        IL6R interleukin 6 receptor
#>   2082            38 ENSG00000164855    TMEM184A transmembrane protei..
#>   2572         60798 ENSG00000080503     SMARCA2 SWI/SNF related, mat..
#>   3421         -3045 ENSG00000245532       NEAT1 nuclear paraspeckle ..
#>   4709             0 ENSG00000170004        CHD3 chromodomain helicas..
#>    ...           ...               ...         ...                   ...
#>   5999           595 ENSG00000012211    PRICKLE3 prickle planar cell ..
#>   6001             0 ENSG00000082458        DLG3 discs large MAGUK sc..
#>   6006          5335 ENSG00000157514     TSC22D3 TSC22 domain family ..
#>   6007          4728 ENSG00000157514     TSC22D3 TSC22 domain family ..
#>   6020         24279 ENSG00000102181      CD99L2   CD99 molecule like 2
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
## Evaluate calls on chromosome 1
head(as.data.frame(epiRomics_putative_enhanceosome_regulome_active@annotations)[as.data.frame(epiRomics
    "chr1", ])
```

	seqnames	starts	end	width	strand	fold_2	fold_4	fold_8	fold_16	fold_32	fold_64	fold_128	ChIP_name	Histone	gene_chr	gene_start	gene_end	gene_length	gene_strand	gene_id	transcript_id	distanceToTSS	ENSEMBL	SYMBOL	GENENAME
456	chr1	154414784	154414784	1	2	2	1	2	1	2	1	0	Intron (ENST00000622330.4/3570, intron 1 of 6)	1	154429459	154429459	3570	ENST00000622330.4	ENSG00000160712.12	9659	ENSG00000160712	IL6R	interleukin 6 receptor
82	chr1	106838276	106838276	1	0	1	1	2	2	7	1	0	Intron (ENST00000377022.8/54897, intron 4 of 20)	1	106873172	106873172	54897	ENST00000377022.8	ENSG00000130940.14	30940	ENSG00000130940	ZNF326	zinc finger 326
46	chr17	57572879*	57572879*	1	1	1	1	1	1	6	1	0	Intron (ENST00000303635.12/23261, intron 6 of 22)	1	77367	77367	23261	ENST00000303635.12	ENSG00000071735.15	161929	ENSG00000071735	TCF20	transcription activator 1
47	chr17	57574554*	57574554*	1	1	1	1	1	1	6	1	0	Intron (ENST00000303635.12/23261, intron 6 of 22)	1	77367	77367	23261	ENST00000303635.12	ENSG00000071735.15	161314	ENSG00000071735	TCF20	transcription activator 1

	seqnstartsend widthstrandfoxa2mafbnkx2_2							ChIP_Hits annotation		geneChrgeneStartgeneEndgeneLengthgeneStrand		geneIdtranscriptId	distanceToTSS ENSEMBL SYMBOL GENENAME
49	chr1816982769689*	1	1	1	1	1	1	6	Distal Intergenic	1	82018215123670 1	1027124553900008780C00002714634 31829	inter-genic non-protein coding RNA 1714
50	chr18170811727867*	1	1	1	1	1	1	6	Distal Intergenic	1	82018215123670 1	1027124553900008780C00002714634 30661	inter-genic non-protein coding RNA 1714

```
## Find Index
which(names(epiRomics_putative_enhanceosome_regulome_active@annotations) ==
    82)
#> [1] 7
```

```
epiRomics_track_layer_human(
  epiRomics_putative_enhanceosome_regulome_active,
  epiRomics_index = which(
    names(
      epiRomics_putative_enhanceosome_regulome_active@annotations
    ) == 82
  ),
  epiRomics_dB = epiRomics_dB,
  epiRomics_track_connection = epiRomics_track_connection
)
#> [1] "not empty"
#> [1] 36.1883
#> [1] "not empty"
#> [1] 14.7321
#> [1] "not empty"
#> [1] 5.30505
#> [1] "not empty"
#> [1] 2.40628
```


Chromosome 1
10.65 mb
10.75 mb
10.7 mb
CASZ1
ENST00000344008.5
ENST00000496432.6
ENST00000492173.1
ENST00000478728.2
Alpha_ATAC
Beta_ATAC
Beta_RNA
Alpha_RNA
Putative_Enhancer_Index.7
foxa2
mafb
nkx2_2
nkx6_1
pdx1
h2az

```
## What about a region that overlapped with super enhancers from the
## human islet regulome database?

epiRomics_putative_enhanceosome_regulome_super <- epiRomics_enhanceosome(epiRomics_putative_enhancers_f
    epiRomics_dB)
#> >> preparing features information...      2021-09-28 19:49:24
#> >> identifying nearest features...        2021-09-28 19:49:24
#> >> calculating distance from peak to TSS...   2021-09-28 19:49:26
#> >> assigning genomic annotation...        2021-09-28 19:49:26
#> >> adding gene annotation...          2021-09-28 19:49:42
#> 'select()' returned 1:many mapping between keys and columns
#> >> assigning chromosome lengths           2021-09-28 19:49:43
#> >> done...                     2021-09-28 19:49:43
epiRomics_putative_enhanceosome_regulome_super@annotations
#> GRanges object with 2401 ranges and 19 metadata columns:
#>        seqnames              ranges strand |     foxa2      mafb    nkx2_2
#>           <Rle>           <IRanges>  <Rle> | <integer> <integer> <integer>
#>    166     chr1 154418514-154419684      * |         2         2         1
#>    966     chr9     2242369-2242873      * |         2         1         1
#>   1422    chr11   65416576-65419753      * |         1         1         2
#>     16     chr1   10685395-10688670      * |         1         0         1
#>    764     chr6   30748438-30749427      * |         2         1         1
#>    ...      ...                 ...    ... .       ...       ...       ...
#>   2393    chr22   46103687-46105878      * |         0         0         0
#>   2396    chr22   46108912-46109315      * |         0         0         0
#>   2397    chr22   46109916-46110442      * |         0         0         0
#>   2398    chr22   46115774-46116154      * |         0         0         0
#>   2399    chr22   46116326-46116501      * |         0         0         0
#>           nxk6_1      pdx1      h2az ChIP_Hits             annotation   geneChr
#>        <integer> <integer> <integer> <numeric>            <character> <integer>
#>    166         2         1         2        10 Intron (ENST00000622..         1
#>    966         1         2         1         8      Distal Intergenic         9
#>   1422         1         1         2         8      Distal Intergenic        11
#>     16         1         2         2         7 Intron (ENST00000377..         1
#>    764         1         1         1         7 Intron (ENST00000656..         6
#>    ...       ...       ...       ...       ...                   ...       ...
#>   2393         0         0         0         0 Exon (ENST0000038105..        22
#>   2396         0         0         0         0 Exon (ENST0000043543..        22
#>   2397         0         0         0         0       Promoter (2-3kb)        22
#>   2398         0         0         0         0       Promoter (2-3kb)        22
#>   2399         0         0         0         0       Promoter (2-3kb)        22
#>        geneStart   geneEnd geneLength geneStrand      geneId      transcriptId
#>        <integer> <integer>  <integer>  <integer> <character>       <character>
#>    166 154429343 154449979      20637          1        3570 ENST00000476006.5
#>    966   2181571   2186183       4613          1        6595 ENST00000635392.1
#>   1422  65422798  65445540      22743          1      283131 ENST00000501122.2
#>     16  10660737  10693912      33176          2       54897 ENST00000478728.2
#>    764  30743199  30744547       1349          2        8870 ENST00000259874.6
#>    ...       ...       ...        ...        ...         ...                ...
#>   2393  46112749  46112822         74          1      406883 ENST00000362116.3
#>   2396  46112749  46112822         74          1      406883 ENST00000362116.3
#>   2397  46112749  46112822         74          1      406883 ENST00000362116.3
#>   2398  46113686  46113768         83          1      406884 ENST00000385140.1
```

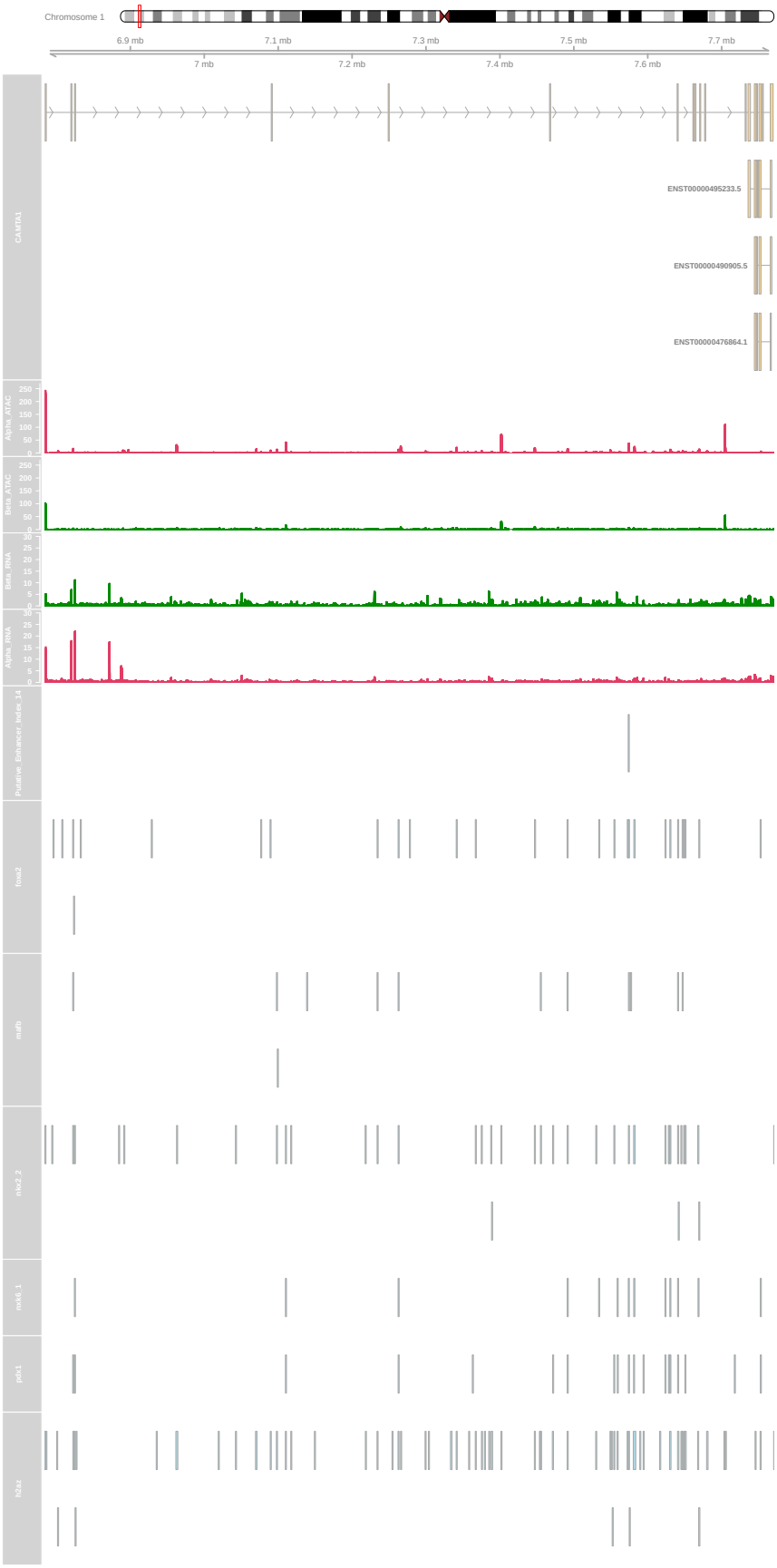
```
#>   2399  46113686  46113768        83         1      406884 ENST00000385140.1
#>        distanceToTSS         ENSEMBL      SYMBOL                GENENAME
#>            <numeric>     <character> <character>             <character>
#>    166         -9659 ENSG00000160712        IL6R interleukin 6 receptor
#>    966         60798 ENSG00000080503     SMARCA2 SWI/SNF related, mat..
#>   1422         -3045 ENSG00000245532       NEAT1 nuclear paraspeckle ..
#>     16          5242 ENSG00000130940       CASZ1   castor zinc finger 1
#>    764         -3891 ENSG00000137331        IER3 immediate early resp..
#>    ...           ...             ...         ...                    ...
#>   2393         -6871 ENSG00000283990   MIRLET7A3      microRNA let-7a-3
#>   2396         -3434 ENSG00000283990   MIRLET7A3      microRNA let-7a-3
#>   2397         -2307 ENSG00000283990   MIRLET7A3      microRNA let-7a-3
#>   2398          2088 ENSG00000284520    MIRLET7B        microRNA let-7b
#>   2399          2640 ENSG00000284520    MIRLET7B        microRNA let-7b
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
## Evaluate calls on chromosome 1
head(as.data.frame(epiRomics_putative_enhanceosome_regulome_super@annotations)[as.data.frame(epiRomics_
    "chr1", ])
```

	seqnames	starts	end	width	strand	fosl2_ab	fosl2_bk	fosl2_xl	jund_ab	jund_bk	jund_xl	atf2_ab	ChIP_number	Histone	geneChr	geneStart	geneEnd	geneLength	geneStrand	transcript	distance	ENSEMBL	SYMBOL	GENENAME
166	chr1	154[illegible]	[illegible]	84	2	2	1	2	1	2	10	Intron (ENST00000622330.4/3570, intron 1 of 6)	1	154[illegible]	[illegible]	3570	ENST00000622330.4	9659	ENSG00000160712	IL6R	interleukin 6 receptor
16	chr1	106[illegible]	[illegible]	76	1	0	1	1	2	2	7	Intron (ENST00000377022.8/54897, intron 4 of 20)	1	106[illegible]	[illegible]	54897	ENST00000377022.8	5242	ENSG00000130940	CASZ1	castor zinc finger 1
1	chr1	757[illegible]	[illegible]	89*	1	1	1	1	1	1	6	Intron (ENST00000303635.12/23261, intron 6 of 22)	1	773[illegible]	[illegible]	23261	ENST00000303635.12	161929	ENSG00000171735	CEBPB	CCAAT enhancer binding protein beta
2	chr1	757[illegible]	[illegible]	55*	1	1	1	1	1	1	6	Intron (ENST00000303635.12/23261, intron 6 of 22)	1	773[illegible]	[illegible]	23261	ENST00000303635.12	161314	ENSG00000171735	CEBPB	CCAAT enhancer binding protein beta

	seqnames	start	end	width	strand	fold_enrichment	log2_enrichment	fdr	p_value	log10_p_value	ChIP_name	Histone	gene_name	gene_chr	gene_start	gene_end	gene_strand	gene_id	transcript_id	distance	ENSEMBL	SYMBOL	GENENAME
3	chr18	16927	16927	1	*	1	1	1	1	1	1	6	Distal Intergenic	1	82018	82018	1	10272	ENSG00000231829	0	ENSG00000231829	RP11-1055B8.7	RP11-1055B8.7 antisense RNA 1
4	chr18	17087	17087	1	*	1	1	1	1	1	1	6	Distal Intergenic	1	82018	82018	1	10272	ENSG00000230661	0	ENSG00000230661	RP11-1055B8.7	RP11-1055B8.7 antisense RNA 1

```
## Find Index
which(names(epiRomics_putative_enhanceosome_regulome_super@annotations) ==
    1)
#> [1] 14
```

```
epiRomics_track_layer_human(
  epiRomics_putative_enhanceosome_regulome_super,
  epiRomics_index = which(
    names(epiRomics_putative_enhanceosome_regulome_super@annotations) == 1
  ),
  epiRomics_dB = epiRomics_dB,
  epiRomics_track_connection = epiRomics_track_connection
)
#> [1] "not empty"
#> [1] 243.743
#> [1] "not empty"
#> [1] 103.706
#> [1] "not empty"
#> [1] 11.3213
#> [1] "not empty"
#> [1] 22.1498
```

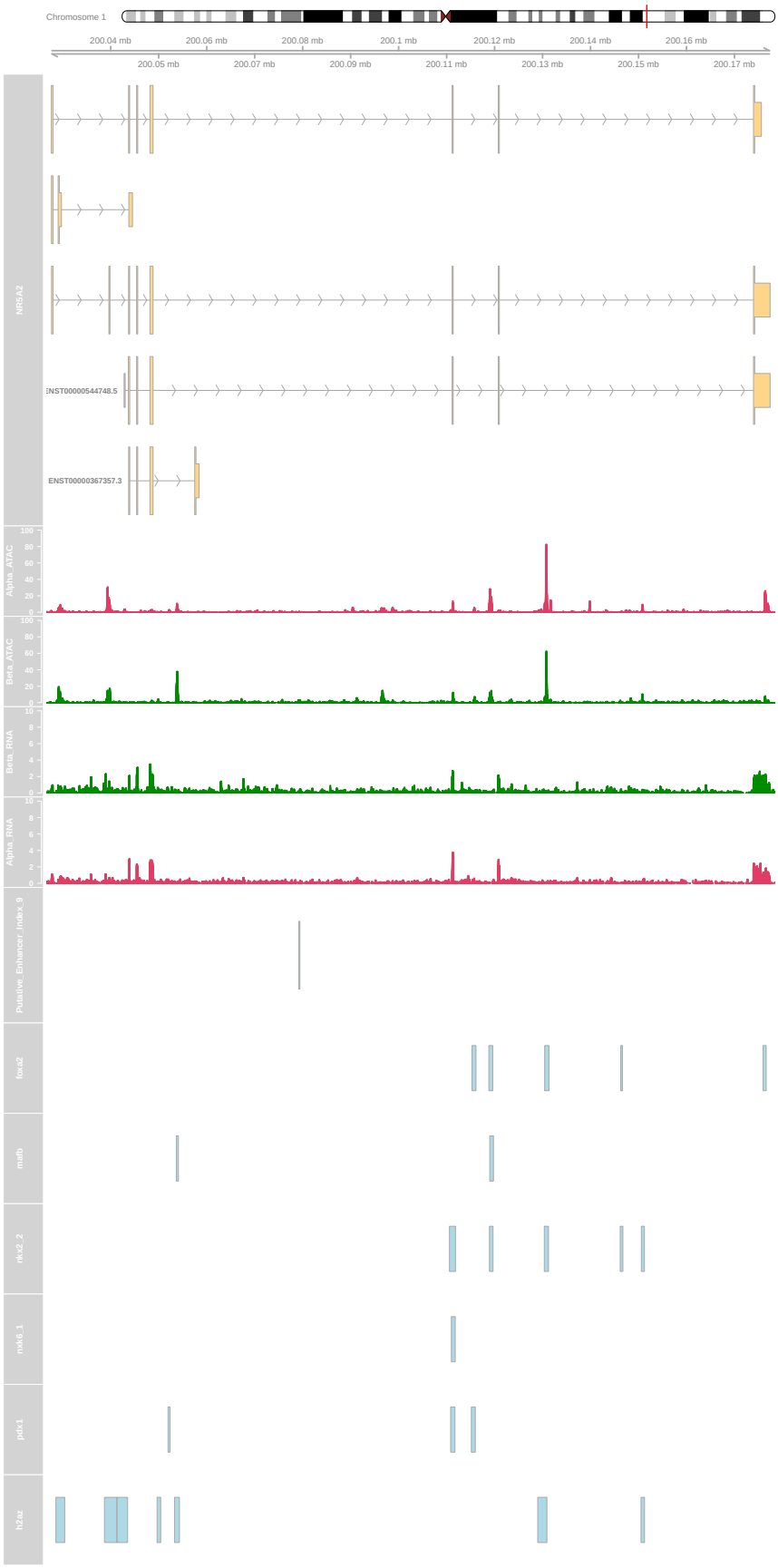

Chromosome 1
6.9 mb
7.1 mb
7.3 mb
7.5 mb
7.7 mb
7 mb
7.2 mb
7.4 mb
7.6 mb
CAMTA1
ENST00000495233.5
ENST00000490905.5
ENST00000476864.1
Alpha_ATAC
Beta_ATAC
Beta_RNA
Alpha_RNA
Putative_Enhancer_Index_14
foxa2
mafb
nkx2_2
nkx6_1
pdx1
h3az

```
## Or, about a region that overlapped with ultra-conserved non coding
## elements?

epiRomics_putative_enhanceosome_ucnes <- epiRomics_enhanceosome(epiRomics_putative_enhancers_filtered_u
    epiRomics_dB)
#> >> preparing features information...      2021-09-28 19:53:08
#> >> identifying nearest features...        2021-09-28 19:53:08
#> >> calculating distance from peak to TSS...   2021-09-28 19:53:09
#> >> assigning genomic annotation...        2021-09-28 19:53:09
#> >> adding gene annotation...          2021-09-28 19:53:20
#> 'select()' returned 1:1 mapping between keys and columns
#> >> assigning chromosome lengths           2021-09-28 19:53:20
#> >> done...                     2021-09-28 19:53:20
epiRomics_putative_enhanceosome_ucnes@annotations
#> GRanges object with 11 ranges and 19 metadata columns:
#>      seqnames              ranges strand |     foxa2      mafb    nkx2_2
#>         <Rle>           <IRanges>  <Rle> | <integer> <integer> <integer>
#>    7     chr9 106921420-106921764      * |         1         1         1
#>    1     chr1 164635220-164635921      * |         0         0         2
#>    6     chr3   71131859-71132164      * |         1         0         0
#>    2     chr1 164711914-164712296      * |         0         0         0
#>    3     chr1 164712350-164713071      * |         0         0         0
#>    8    chr11 114163425-114164860      * |         0         0         0
#>    9    chr15   36903894-36904085      * |         0         0         1
#>   11    chr21   16534340-16534665      * |         0         0         0
#>    4     chr1 200079185-200079426      * |         0         0         0
#>    5     chr1 213585694-213586385      * |         0         0         0
#>   10    chr15   53447393-53447809      * |         0         0         0
#>         nxk6_1      pdx1      h2az ChIP_Hits             annotation   geneChr
#>      <integer> <integer> <integer> <numeric>            <character> <integer>
#>    7         1         0         1         5 Intron (ENST00000472..         9
#>    1         0         1         1         4 Intron (ENST00000420..         1
#>    6         0         0         1         2       Promoter (<=1kb)         3
#>    2         0         0         1         1 Intron (ENST00000420..         1
#>    3         0         0         1         1 Intron (ENST00000420..         1
#>    8         0         0         1         1 Intron (ENST00000335..        11
#>    9         0         0         0         1       Promoter (<=1kb)        15
#>   11         0         0         1         1       Promoter (<=1kb)        21
#>    4         0         0         0         0 Intron (ENST00000236..         1
#>    5         0         0         0         0     Distal Intergenic          1
#>   10         0         0         0         0 Intron (ENST00000662..        15
#>      geneStart   geneEnd geneLength geneStrand      geneId      transcriptId
#>      <integer> <integer>  <integer>  <integer> <character>       <character>
#>    7 106926925 106932462       5538          1       58499 ENST00000480607.5
#>    1 164630981 164799889     168909          1        5087 ENST00000482110.5
#>    6  70959237  71132099     172863          2       27086 ENST00000650188.1
#>    2 164772912 164807571      34660          1        5087 ENST00000558837.5
#>    3 164772912 164807571      34660          1        5087 ENST00000558837.5
#>    8 114180766 114247296      66531          1        7704 ENST00000545851.5
#>    9  36894784  36904067       9284          2        4212 ENST00000559408.1
#>   11  16534952  16607137      72186          1      388815 ENST00000654245.1
#>    4 200043810 200058424      14615          1        2494 ENST00000367357.3
#>    5 213832591 213841041       8451          2   100505832 ENST00000609394.5
```

```
#>   10  53513742  53541080      27339          2      256764 ENST00000614174.4
#>      distanceToTSS         ENSEMBL      SYMBOL              GENENAME
#>          <numeric>     <character> <character>           <character>
#>    7         -5161 ENSG00000148143      ZNF462 zinc finger protein ..
#>    1          4239 ENSG00000185630        PBX1         PBX homeobox 1
#>    6             0 ENSG00000114861       FOXP1        forkhead box P1
#>    2        -60616 ENSG00000185630        PBX1         PBX homeobox 1
#>    3        -59841 ENSG00000185630        PBX1         PBX homeobox 1
#>    8        -15906 ENSG00000109906      ZBTB16 zinc finger and BTB ..
#>    9             0 ENSG00000134138       MEIS2        Meis homeobox 2
#>   11          -287 ENSG00000215386    MIR99AHG mir-99a-let-7c clust..
#>    4         35375 ENSG00000116833       NR5A2 nuclear receptor sub..
#>    5        254656 ENSG00000230461   PROX1-AS1  PROX1 antisense RNA 1
#>   10         93271 ENSG00000166415       WDR72    WD repeat domain 72
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
```

```
epiRomics_track_layer_human(
  epiRomics_putative_enhanceosome_ucnes,
  epiRomics_index = 9,
  epiRomics_dB = epiRomics_dB,
  epiRomics_track_connection = epiRomics_track_connection
)
#> [1] "not empty"
#> [1] 82.795
#> [1] "not empty"
#> [1] 62.847
#> [1] "not empty"
#> [1] 3.55117
#> [1] "not empty"
#> [1] 3.80884
```


Chromosome 1
200.04 mb
200.06 mb
200.08 mb
200.1 mb
200.12 mb
200.14 mb
200.16 mb
200.05 mb
200.07 mb
200.09 mb
200.11 mb
200.13 mb
200.15 mb
200.17 mb
NR6A2
ENST00000544748.5
ENST00000367357.3
Alpha_ATAC
Beta_ATAC
Beta_RNA
Alpha_RNA
Putative_Enhancer_Index_9
foxa2
mafb
nkx2_2
nkx6_1
pdx1
h2az

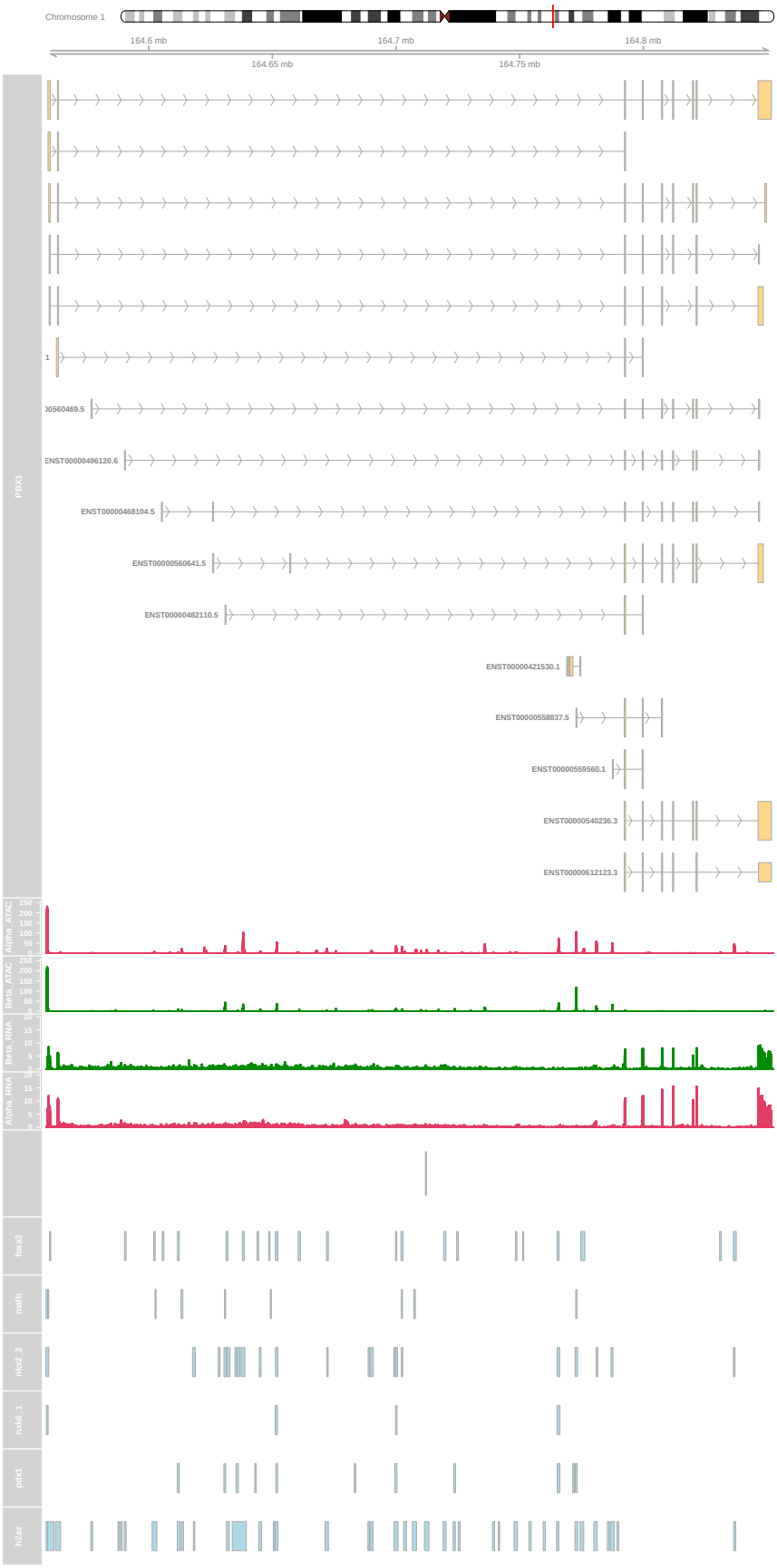
```
## How about applying multiple filters to further increase the confidence of calls?

epiRomics_putative_enhancers_filtered_stringent <-
  epiRomics_enhancers_filter(
    epiRomics_enhancers_filter(
      epiRomics_enhancers_filter(
        epiRomics_enhancers_filter(epiRomics_putative_enhancers, epiRomics_dB,
          epiRomics_type =
            "hg38_custom_fantom"
        ),
        epiRomics_dB,
        epiRomics_type = "hg38_custom_regulome_active"
      ),
      epiRomics_dB,
      epiRomics_type = "hg38_custom_regulome_super"
    ),
    epiRomics_dB,
    epiRomics_type = "hg38_custom_ucnes"
  )


## Here, we see a highly conservative list of putative enhancer calls that overlap with four different
epiRomics_putative_enhancers_filtered_stringent@annotations
#> GRanges object with 2 ranges and 0 metadata columns:
#>       seqnames              ranges strand
#>          <Rle>           <IRanges>  <Rle>
#>   [1]     chr1 164711914-164712296      *
#>   [2]     chr1 164712350-164713071      *
#>   -------
#>   seqinfo: 595 sequences (1 circular) from hg38 genome
epiRomics_putative_enhanceosome_stringent <-
  epiRomics_enhanceosome(
    epiRomics_putative_enhancers_filtered_stringent,
    epiRomics_dB
  )
#> >> preparing features information...      2021-09-28 19:55:53
#> >> identifying nearest features...        2021-09-28 19:55:53
#> >> calculating distance from peak to TSS...   2021-09-28 19:55:54
#> >> assigning genomic annotation...        2021-09-28 19:55:54
#> >> adding gene annotation...          2021-09-28 19:56:06
#> 'select()' returned 1:1 mapping between keys and columns
#> >> assigning chromosome lengths           2021-09-28 19:56:06
#> >> done...                     2021-09-28 19:56:06
```

```
epiRomics_track_layer_human(
  epiRomics_putative_enhanceosome_stringent,
  epiRomics_index = 1,
  epiRomics_dB = epiRomics_dB,
  epiRomics_track_connection = epiRomics_track_connection
)
#> [1] "not empty"
#> [1] 233.705
#> [1] "not empty"
```

```
#> [1] 221.083
#> [1] "not empty"
#> [1] 9.31751
#> [1] "not empty"
#> [1] 15.8259
```


Chromosome 1
164.6 mb
164.7 mb
164.8 mb
164.65 mb
164.75 mb
PBX1
ENST00000496120.6
ENST00000468104.5
ENST00000560641.5
ENST00000482110.5
ENST00000421530.1
ENST00000558837.5
ENST00000559560.1
ENST00000540236.3
ENST00000612123.3
Alpha_ATAC
Beta_ATAC
Beta_RNA
Alpha_RNA
foxa2
nsfb
nkx2_2
nxk6_1
pdx1
h2az

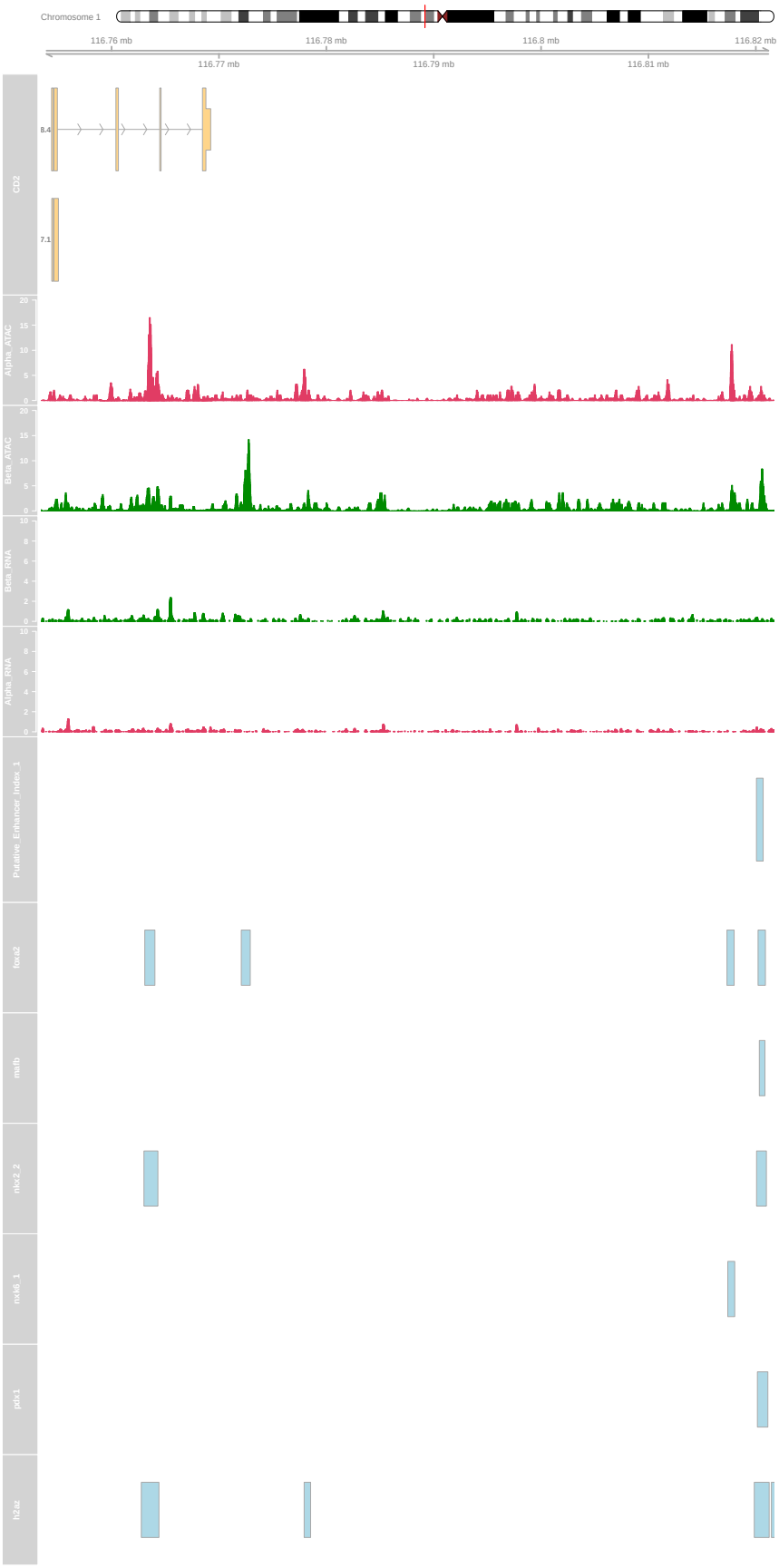
```
## How can we use these putative enhanceosome regions to infer
## biology between cell states? In this example, we will integrate
## ATAC-Seq data differential testing showing differences in
## chromatin accessibility between alpha and beta cells

## Read differentially binding data generated with DiffBind comparing
## human alpha and beta cell chromatin
b.v.a <- read.csv(system.file("extdata", "DBA_Beta_Versus_Alpha.csv", package = "epiRomics"))
b.v.a <- GRanges(b.v.a)

# Filter for beta enriched chromatin regions
beta.enriched <- b.v.a[b.v.a$Fold >= 1, ]

# Connect to our putative enhanceosomes
beta_enhancer_regions <- epiRomics_regions_of_interest(epiRomics_putative_enhanceosome_fantom,
    beta.enriched)
```

```
## Now, lets visualize the top candidate region we found after connecting our differential chromatin an
epiRomics_track_layer_human(
  beta_enhancer_regions,
  epiRomics_index = 1,
  epiRomics_dB = epiRomics_dB,
  epiRomics_track_connection = epiRomics_track_connection
)
#> [1] "not empty"
#> [1] 16.5139
#> [1] "not empty"
#> [1] 14.2229
#> [1] "not empty"
#> [1] 2.38379
#> [1] "not empty"
#> [1] 1.29041
```


Chromosome 1
116.76 mb
116.77 mb
116.78 mb
116.79 mb
116.8 mb
116.81 mb
116.82 mb
CD2
8.4
7.1
Alpha_ATAC
Beta_ATAC
Beta_RNA
Alpha_RNA
Putative_Enhancer_Index_1
foxi2
nafb
nkx2_2
nkx6_1
pitx1
h2az

Session Information

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

```
sessionInfo()
#> R version 4.1.1 (2021-08-10)
#> Platform: x86_64-apple-darwin17.0 (64-bit)
#> Running under: macOS Catalina 10.15.7
#>
#> Matrix products: default
#> BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
#> LAPACK: /Library/Frameworks/R.framework/Versions/4.1/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] stats4    parallel  stats     graphics  grDevices utils     datasets
#> [8] methods   base
#>
#> other attached packages:
#>  [1] BSgenome.Hsapiens.UCSC.hg38_1.4.3
#>  [2] BSgenome_1.60.0
#>  [3] rtracklayer_1.52.1
#>  [4] Biostrings_2.60.2
#>  [5] XVector_0.32.0
#>  [6] org.Hs.eg.db_3.13.0
#>  [7] TxDb.Hsapiens.UCSC.hg38.knownGene_3.13.0
#>  [8] GenomicFeatures_1.44.2
#>  [9] AnnotationDbi_1.54.1
#> [10] Biobase_2.52.0
#> [11] GenomicRanges_1.44.0
#> [12] GenomeInfoDb_1.28.4
#> [13] IRanges_2.26.0
#> [14] S4Vectors_0.30.0
#> [15] BiocGenerics_0.38.0
#> [16] epiRomics_0.1.3
#>
#> loaded via a namespace (and not attached):
#>   [1] utf8_1.2.2
#>   [2] tidyselect_1.1.1
#>   [3] RSQLite_2.2.8
#>   [4] htmlwidgets_1.5.4
#>   [5] grid_4.1.1
#>   [6] BiocParallel_1.26.2
#>   [7] scatterpie_0.1.7
#>   [8] munsell_0.5.0
#>   [9] codetools_0.2-18
#>  [10] withr_2.4.2
#>  [11] colorspace_2.0-2
#>  [12] GOSemSim_2.18.1
#>  [13] filelock_1.0.2
#>  [14] highr_0.9
#>  [15] knitr_1.34
```

```
#>  [16] rstudioapi_0.13
#>  [17] DOSE_3.18.2
#>  [18] MatrixGenerics_1.4.3
#>  [19] GenomeInfoDbData_1.2.6
#>  [20] polyclip_1.10-0
#>  [21] bit64_4.0.5
#>  [22] farver_2.1.0
#>  [23] treeio_1.16.2
#>  [24] vctrs_0.3.8
#>  [25] generics_0.1.0
#>  [26] TH.data_1.0-10
#>  [27] xfun_0.26
#>  [28] biovizBase_1.40.0
#>  [29] BiocFileCache_2.0.0
#>  [30] party_1.3-8
#>  [31] regioneR_1.24.0
#>  [32] R6_2.5.1
#>  [33] graphlayouts_0.7.1
#>  [34] AnnotationFilter_1.16.0
#>  [35] gridGraphics_0.5-1
#>  [36] bitops_1.0-7
#>  [37] cachem_1.0.6
#>  [38] fgsea_1.18.0
#>  [39] DelayedArray_0.18.0
#>  [40] assertthat_0.2.1
#>  [41] promises_1.2.0.1
#>  [42] BiocIO_1.2.0
#>  [43] scales_1.1.1
#>  [44] vroom_1.5.5
#>  [45] multcomp_1.4-17
#>  [46] ggraph_2.0.5
#>  [47] nnet_7.3-16
#>  [48] enrichplot_1.13.1.992
#>  [49] gtable_0.3.0
#>  [50] tidygraph_1.2.0
#>  [51] sandwich_3.0-1
#>  [52] ensembldb_2.16.4
#>  [53] rlang_0.4.11
#>  [54] splines_4.1.1
#>  [55] lazyeval_0.2.2
#>  [56] dichromat_2.0-0
#>  [57] checkmate_2.0.0
#>  [58] BiocManager_1.30.16
#>  [59] yaml_2.2.1
#>  [60] reshape2_1.4.4
#>  [61] backports_1.2.1
#>  [62] httpuv_1.6.3
#>  [63] qvalue_2.24.0
#>  [64] Hmisc_4.5-0
#>  [65] tools_4.1.1
#>  [66] ggplotify_0.1.0
#>  [67] ggplot2_3.3.5
#>  [68] gplots_3.1.1
```

```
#>  [69] ellipsis_0.3.2
#>  [70] RColorBrewer_1.1-2
#>  [71] Rcpp_1.0.7
#>  [72] plyr_1.8.6
#>  [73] base64enc_0.1-3
#>  [74] progress_1.2.2
#>  [75] zlibbioc_1.38.0
#>  [76] purrr_0.3.4
#>  [77] RCurl_1.98-1.5
#>  [78] prettyunits_1.1.1
#>  [79] rpart_4.1-15
#>  [80] viridis_0.6.1
#>  [81] zoo_1.8-9
#>  [82] SummarizedExperiment_1.22.0
#>  [83] ggrepel_0.9.1
#>  [84] cluster_2.1.2
#>  [85] magrittr_2.0.1
#>  [86] data.table_1.14.0
#>  [87] DO.db_2.9
#>  [88] mvtnorm_1.1-2
#>  [89] ProtGenerics_1.24.0
#>  [90] matrixStats_0.61.0
#>  [91] patchwork_1.1.1
#>  [92] hms_1.1.0
#>  [93] mime_0.11
#>  [94] evaluate_0.14
#>  [95] xtable_1.8-4
#>  [96] XML_3.99-0.8
#>  [97] jpeg_0.1-9
#>  [98] gridExtra_2.3
#>  [99] compiler_4.1.1
#> [100] biomaRt_2.48.3
#> [101] tibble_3.1.4
#> [102] KernSmooth_2.23-20
#> [103] shadowtext_0.0.9
#> [104] crayon_1.4.1
#> [105] htmltools_0.5.2
#> [106] ggfun_0.0.4
#> [107] later_1.3.0
#> [108] tzdb_0.1.2
#> [109] Formula_1.2-4
#> [110] aplot_0.1.1
#> [111] tidyr_1.1.3
#> [112] libcoin_1.0-8
#> [113] DBI_1.1.1
#> [114] tweenr_1.0.2
#> [115] formatR_1.11
#> [116] ChIPseeker_1.28.3
#> [117] dbplyr_2.1.1
#> [118] MASS_7.3-54
#> [119] rappdirs_0.3.3
#> [120] boot_1.3-28
#> [121] Matrix_1.3-4
```

```
#> [122] readr_2.0.1
#> [123] Gviz_1.36.2
#> [124] igraph_1.2.6
#> [125] pkgconfig_2.0.3
#> [126] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
#> [127] GenomicAlignments_1.28.0
#> [128] coin_1.4-1
#> [129] foreign_0.8-81
#> [130] xml2_1.3.2
#> [131] ggtree_3.0.4
#> [132] yulab.utils_0.0.2
#> [133] stringr_1.4.0
#> [134] VariantAnnotation_1.38.0
#> [135] digest_0.6.28
#> [136] strucchange_1.5-2
#> [137] rmarkdown_2.11
#> [138] fastmatch_1.1-3
#> [139] tidytree_0.3.5
#> [140] htmlTable_2.2.1
#> [141] annotatr_1.18.1
#> [142] restfulr_0.0.13
#> [143] curl_4.3.2
#> [144] gtools_3.9.2
#> [145] shiny_1.7.0
#> [146] Rsamtools_2.8.0
#> [147] modeltools_0.2-23
#> [148] rjson_0.2.20
#> [149] jsonlite_1.7.2
#> [150] nlme_3.1-153
#> [151] lifecycle_1.0.1
#> [152] viridisLite_0.4.0
#> [153] fansi_0.5.0
#> [154] pillar_1.6.2
#> [155] lattice_0.20-45
#> [156] plotrix_3.8-2
#> [157] KEGGREST_1.32.0
#> [158] fastmap_1.1.0
#> [159] httr_1.4.2
#> [160] survival_3.2-13
#> [161] GO.db_3.13.0
#> [162] interactiveDisplayBase_1.30.0
#> [163] glue_1.4.2
#> [164] png_0.1-7
#> [165] BiocVersion_3.13.1
#> [166] bit_4.0.4
#> [167] ggforce_0.3.3
#> [168] stringi_1.7.4
#> [169] blob_1.2.2
#> [170] AnnotationHub_3.0.1
#> [171] caTools_1.18.2
#> [172] latticeExtra_0.6-29
#> [173] memoise_2.0.0
#> [174] dplyr_1.0.7
```

```
#> [175] ape_5.5
```