

Sushi: An R/Bioconductor package for visualizing genomic data

Douglas H. Phanstiel* Alan P. Boyle, Carlos L. Araya, and Mike Snyder

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1 Introduction

Sushi is an R package for plotting genomic data stored in multiple common genomic formats including bed, bedpe, bedgraph format. The package was

*Corresponding contact: doug.phanstiel@gmail.com

designed to be very flexible to allow for combinations of plots into multipanel figures that can include plots made by Sushi, R basecode, or other R packages. Sushi allows for simple flexible plotting of gene structures, transcript structures, sequencing tracks, ChIP-seq peaks, chromatin interactions, GWAS results and other common genomic data types. This vignette shows some examples of the functions included in Sushi to get you started with plotting these diverse data types.

2 Data

2.1 Data types

Sushi accepts 4 types of genomic data as input. These include:

- bed format: 3-6 columns (chromosome, start, stop, name, score, strand)
- bedpe format: 6-10 columns (chromosome1, start1, stop1, chromosome2, start2, stop2, name, score, strand1, strand2)
- bedgraph format: 4 columns (chromosome, start, stop, score)
- interaction matrix: This is matrix in which row and column names are genomic coordinates and matrix values are some type of interaction score.

** strands can be represented as 1 or -1 or "+" and "-".

** Some functions may require additional information depending on the plot and features desired.

2.2 Example datasets

To illustrate how Sushi works, we have included several publicly available data sets in the package Sushi. The data types include RNA-seq, ChIP-seq, ChIA-PET, and HiC data:

Sushi_5C.bedpe	Sanyal et al. [7]
Sushi_ChIAPET_pol2.bedpe	Li et al. [4]
Sushi_ChIPExo_CTCF.bedgraph	Rhee and Pugh [6]
Sushi_ChIPSeq_CTCF.bedgraph	The ENCODE Project Consortium [8]
Sushi_ChIPSeq_pol2.bed	The ENCODE Project Consortium [8]
Sushi_ChIPSeq_pol2.bedgraph	The ENCODE Project Consortium [8]
Sushi_ChIPSeq_severalfactors.bed	The ENCODE Project Consortium [8]
Sushi_DNaseI.bedgraph	Neph et al. [5]
Sushi_GWAS.bed	International Consortium for Blood Pressure [3]
Sushi_HiC.matrix	Dixon et al. [2]
Sushi_RNASeq_K562.bedgraph	The ENCODE Project Consortium [8]
Sushi_genes.bed	Biomart [1]
Sushi_hg18_genome	Biomart [1]
Sushi_transcripts.bed	The ENCODE Project Consortium [8]

These data sets can be loaded using the following commands:

```
> library('Sushi')
> Sushi_data = data(package = 'Sushi')
> data(list = Sushi_data$results[,3])
```

To see which data sets are loaded

```
> Sushi_data$results[,3]

[1] "Sushi_5C.bedpe"                      "Sushi_ChIAPET_pol2.bedpe"
[3] "Sushi_ChIPExo_CTCF.bedgraph"          "Sushi_ChIPSeq_CTCF.bedgraph"
[5] "Sushi_ChIPSeq_pol2.bed"                "Sushi_ChIPSeq_pol2.bedgraph"
[7] "Sushi_ChIPSeq_severalfactors.bed"      "Sushi_DNaseI.bedgraph"
[9] "Sushi_GWAS.bed"                       "Sushi_HiC.matrix"
[11] "Sushi_RNASeq_K562.bedgraph"           "Sushi_genes.bed"
[13] "Sushi_hg18_genome"                   "Sushi_transcripts.bed"
```

3 Functions

3.1 Functions overview

Sushi functions can be broken down into 3 categories: plotting, annotating, zooming, and coloring. Plotting functions generate a basic plot object using the data. Annotating functions add information to the plots such as an x-axis labeling the genomic region or a legend describing the values represented by different colors. Zooming functions allow for highlighting and zooming of genomic regions, which are of particular use for multipanel plots generated with base R functions `mfrow()` or `layout()`. The coloring functions provide simple tools for generating R colors and palettes.

- Plotting functions: `plotBed()`, `plotBedgraph()`, `plotBedpe()`, `plotGenes()`, `plotHiC()`, and `plotManhattan()`
- Annotating functions: `labelgenome()` and `addlegend()`
- Zooming functions: `zoomsregion()` and `zoombox()`
- Coloring functions: `maptocolors()`, `SushiColors()`, and `opaque()`

3.2 Non-Sushi Functions

An important characteristic of Sushi plots is their compatibility with all base R functions and their ability to be combined into complex multipanel figures. Two of the most useful base R functions for creating multipanel figures are `layout()` and `mfrow()`. Basic R plotting functions such as `axis()`, `mttext()`, and `legend()` are also particularly well suited to combine with Sushi plots. A familiarity with these functions will greatly improve your ability to create Sushi plots.

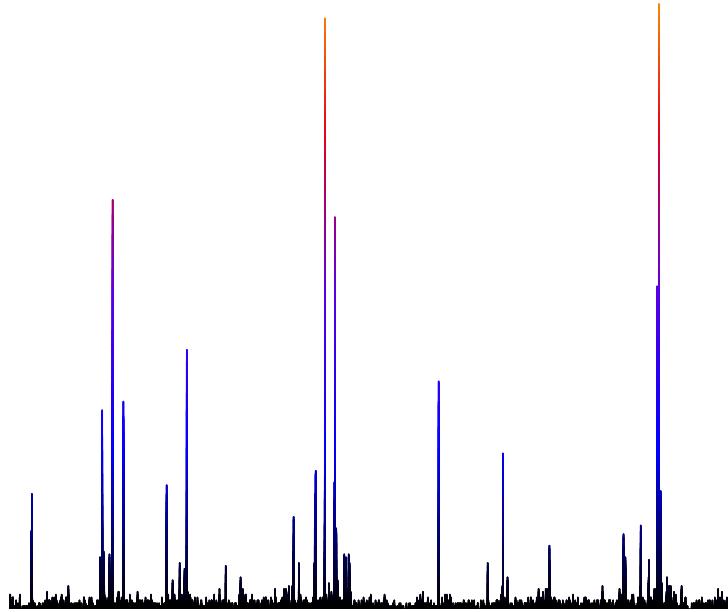
3.3 plotBedgraph

Signal tracks can be plotted using `plotBedgraph()`. The input requires data in bedgraph format. We will demonstrate this using bedgraph data representing a DNaseI hypersensitivity experiment in K562 cells.

```
> head(Sushi_DNaseI.bedgraph)
      chrom start   end value
1 chr11 77224 77244     1
2 chr11 77244 77384     2
3 chr11 96704 96724     1
4 chr11 96724 96844     3
5 chr11 96844 96884     2
6 chr11 97904 97924     3
```

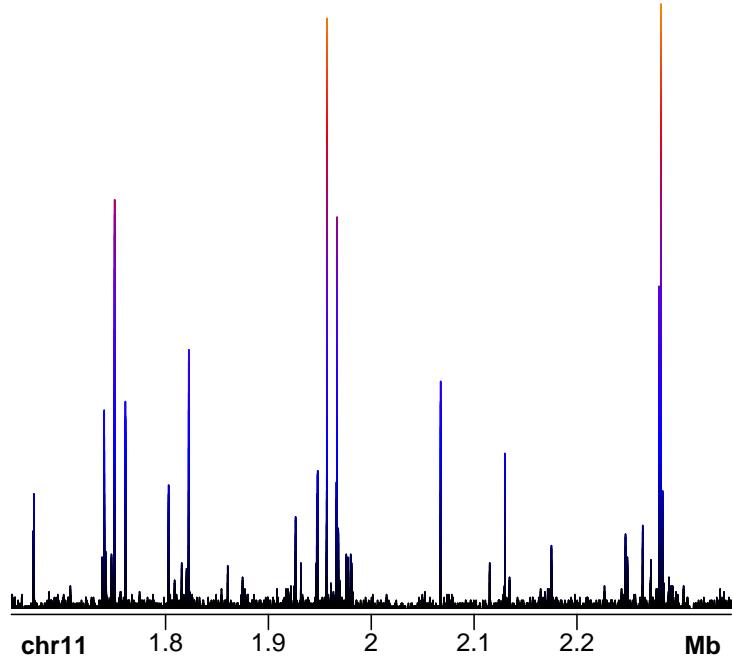
The `plotBedgraph()` function is used to plot the data. As with most Sushi functions the basic required arguments include the data to be plotted, the chromosome, and a start and stop position.

```
> chrom          = "chr11"
> chromstart    = 1650000
> chromend      = 2350000
> plotBedgraph(Sushi_DNaseI.bedgraph,chrom,chromstart,chromend,colorbycol= SushiColors(5))
```



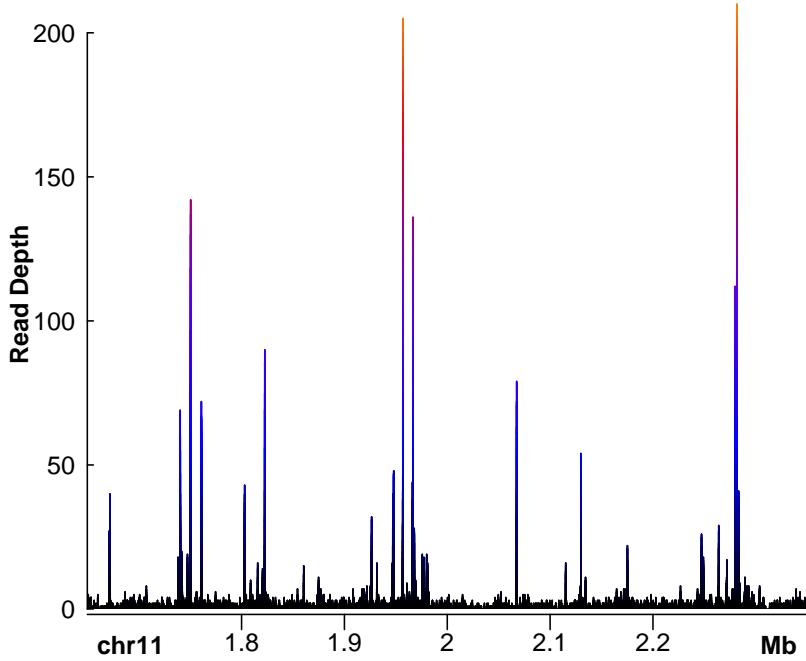
To annotate the genome position we use the `labelgenome()` function. We us `n = 4` to specify the desired number of tickmarks. The scale is set to Mb (other options are Kb or bp).

```
> labelgenome(chrom, chromstart, chromend, n=4, scale="Mb")
```



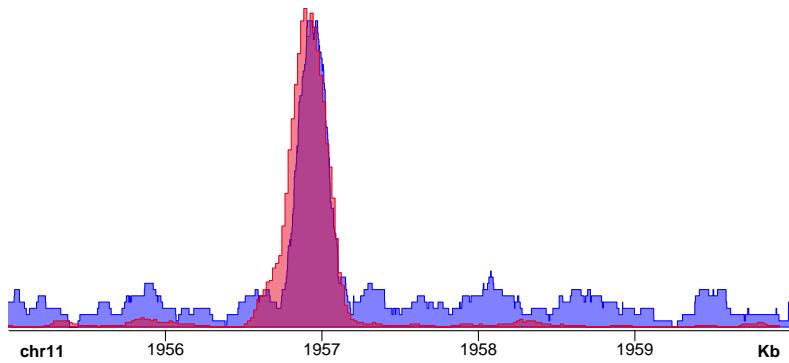
The y-axis can be added using basic R functions `mtext()` and `axis()`.

```
> mtext("Read Depth", side=2, line=1.75, cex=1, font=2)
> axis(side=2, las=2, tcl=.2)
```



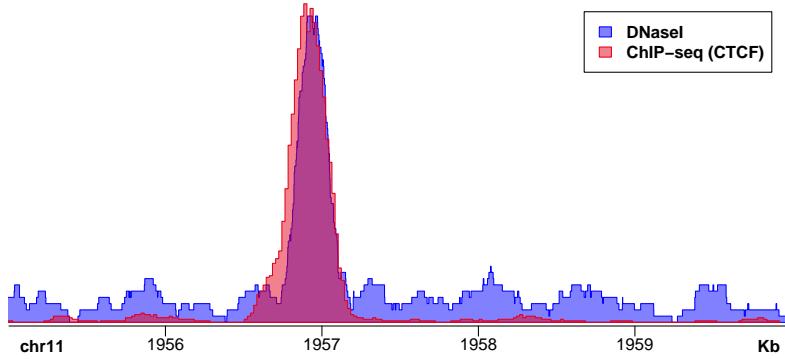
Multiple bedgraph tracks can be plotted on the same plot by setting `overlay=TRUE`. Transparency can be added for easier viewing by adjusting the transparency value. The second plot can be rescaled to the maximum of the first plot by setting `rescaleoverlay=TRUE`.

```
> chrom          = "chr11"
> chromstart    = 1955000
> chromend      = 1960000
> plotBedgraph(Sushi_ChIPSeq_CTCF.bedgraph,chrom,chromstart,chromend,
               transparency=.50,color=SushiColors(2)(2)[1])
> plotBedgraph(Sushi_DNaseI.bedgraph,chrom,chromstart,chromend,
               transparency=.50,color=SushiColors(2)(2)[2],overlay=TRUE,
               rescaleoverlay=TRUE)
> labelgenome(chrom,chromstart,chromend,n=3,scale="Kb")
```



Then we can use the base R function `legend()` to add a legend to the plot. First we need to use the `rgb` function to add transparency to the colors in order to match out plot.

```
> legend("topright", inset=0.025, legend=c("DNaseI", "ChIP-seq (CTCF)",  
    fill=opaque(SushiColors(2)(2)), border=SushiColors(2)(2), text.font=2,  
    cex=1.0)
```

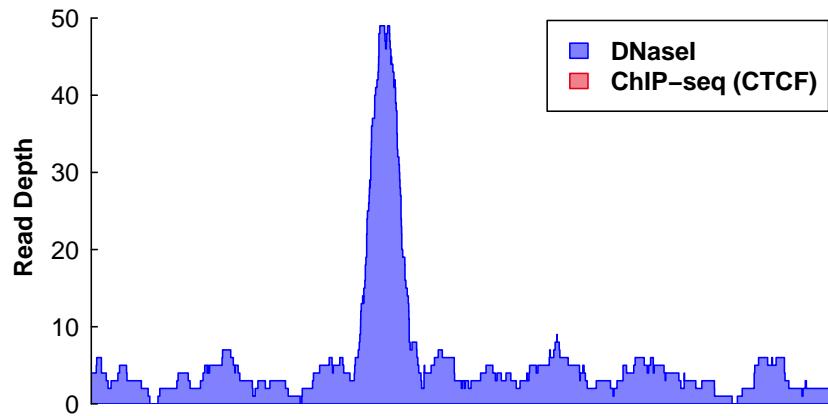


Setting `flip=TRUE` is another method that can be used to compare tracks. First, we will use `mfrow` to divided the plotting device into two vertically stacked regions.

```
> par(mfrow=c(2,1), mar=c(1,4,1,1))
```

Next, we plot the first plot. We set the transparency of the plot to 0.5. We will also add the legend.

```
> plotBedgraph(Sushi_ChIPSeq_CTCF.bedgraph, chrom, chromstart, chromend, transparency=.50,
               color=SushiColors(2)(2)[1])
> axis(side=2, las=2, tcl=.2)
> mtext("Read Depth", side=2, line=1.75, cex=1, font=2)
> legend("topright", inset=0.025, legend=c("DNaseI", "ChIP-seq (CTCF)"),
         fill=opaque(SushiColors(2)(2)), border=SushiColors(2)(2), text.font=2,
         cex=1.0)
```



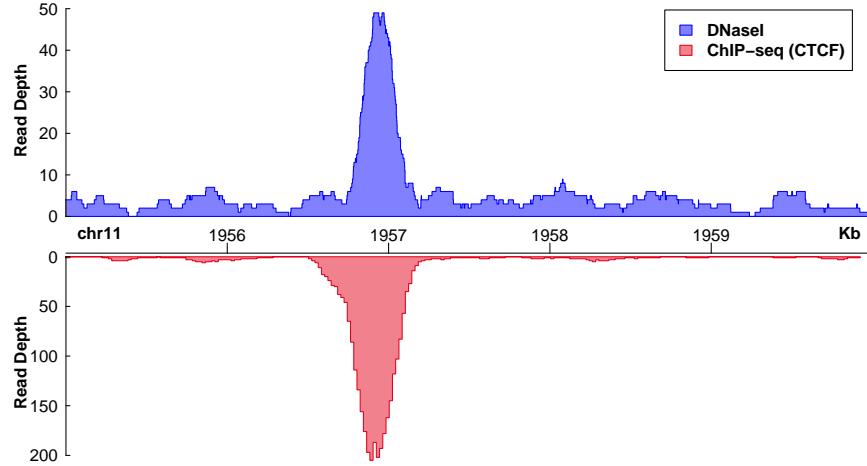
Finally, we add the second plot with `flip=TRUE`. We will also label the x-axis using `labelgenome()` and label the y-axis using `mtext()` and `axis()`.

```
> plotBedgraph(Sushi_DNaseI.bedgraph, chrom, chromstart, chromend,
               transparency=.50, flip=TRUE, color=SushiColors(2)(2)[2])
> labelgenome(chrom, chromstart, chromend, side=3, n=3, scale="Kb")
```

```

> axis(side=2,las=2,tcl=.2,at=pretty(par("yaxp") [c(1,2)]),
      labels=-1*pretty(par("yaxp") [c(1,2)]))
> mtext("Read Depth",side=2,line=1.75,cex=1,font=2)

```



3.4 plotHic

HiC interaction plots can be plotted given an interaction matrix in which row and column names are genomic coordinates and matrix values are some type of interaction score.

```

> Sushi_HiC.matrix[100:105,100:105]

 3980000 4020000 4060000 4100000 4140000 4180000
3980000 0.000000 50.087965 49.689032 22.89760 7.438259 2.219527
4020000 50.087965 40.469337 33.922805 24.07214 12.652542 3.620466
4060000 49.689032 33.922805 26.998026 30.17873 21.879022 6.850893
4100000 22.897599 24.072145 30.178735 54.47335 48.570924 11.379299
4140000 7.438259 12.652542 21.879022 48.57092 45.265394 26.369969
4180000 2.219527 3.620466 6.850893 11.37930 26.369969 11.413106

```

The `plotHic()` function is used to plot the data while the `labelgenome()` function is used to add the genome labels to the x-axis. `plotHic()` returns an object indicating the color palette and data range that can be fed into `addlegend()` to create a legend.

```

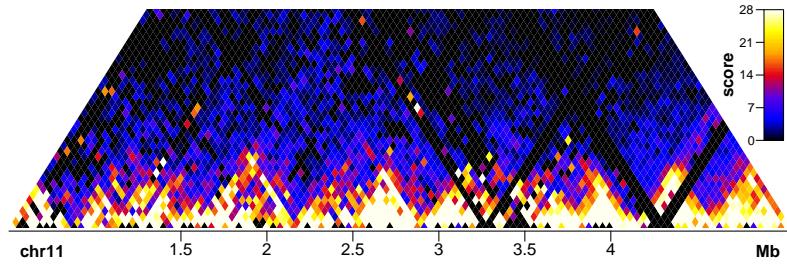
> chrom          = "chr11"
> chromstart     = 500000
> chromend       = 5050000
> phic = plotHic(Sushi_HiC.matrix, chrom,chromstart, chromend, max_y = 20,
                  zrange=c(0,28), palette=SushiColors(7))

```

```

> addlegend(phiic[[1]], palette=phiic[[2]], title="score", side="right",
            bottominset=0.4, topinset=0, xoffset=-.035, labelside="left",
            width=0.025, title.offset=0.035)
> labelgenome(chrom, chromstart, chromend, n=4, scale="Mb",
              edgeblankfraction=0.20)

```



`plotHic()` has a number of customizable options. The plot can be flipped over the x-axis by setting `flip = TRUE`. The color palette can be changed by the `palette` argument.

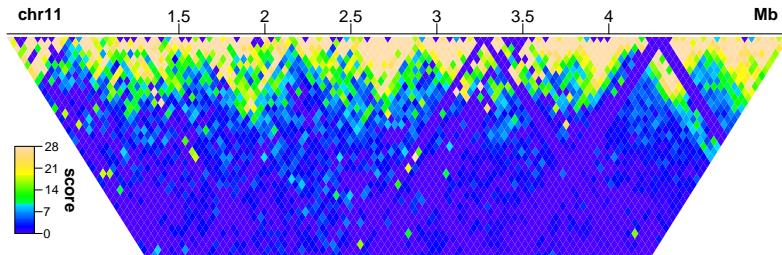
`addlegend()` also has customizable features. The legend can be moved to the left side of the plot by setting `side = "left"` and the labeling can be moved to the right side of the legend by setting `labelside = "right"`. The vertical position of the legend can be adjusted by changing the `topinset` and `bottominset`.

Finally, the x-axis label can be moved to the top of the plot by setting `side = 3` in the `labelgenome()` function.

```

> chrom          = "chr11"
> chromstart     = 500000
> chromend       = 5050000
> phiic = plotHic(Sushi_HiC.matrix,chrom,chromstart,chromend,max_y = 20,
                   zrange=c(0,28),flip=TRUE,palette=topo.colors)
> addlegend(phiic[[1]],palette=phiic[[2]],title="score",side="left",bottominset=0.1,
            topinset=0.5,xoffset=-.035,labelside="right",width=0.025,title.offset=0.035)
> labelgenome(chrom,chromstart,chromend,side=3,n=4,scale="Mb",edgeblankfraction=0.20)

```



3.5 plotBedpe

`plotBedpe()` allows for data in bedpe format to be plotted in multiple fashions. To illustrate this we will use 5C data formatted in the following way.

```
> head(Sushi_5C.bedpe)

  chrom1      start1      end1 chrom2      start2      end2 name    score strand1
1  chr2 234208447 234223064  chr2 234156762 234159135  NA 44.39862 .
2  chr15 41711734 41718116  chr15 41802421 41808201  NA 20.62534 .
3  chr11 64172456 64183193  chr11 64068878 64079209  NA 16.91630 .
4  chr2 234208447 234223064  chr2 234163674 234170252  NA 12.34501 .
5  chr6 41755186 41769245  chr6 41435903 41452283  NA 11.63480 .
6  chr11 64159283 64172456  chr11 64068878 64079209  NA 11.13098 .

  strand2 samplenumber
1          .
2          .
3          .
4          .
5          .
6          .
```

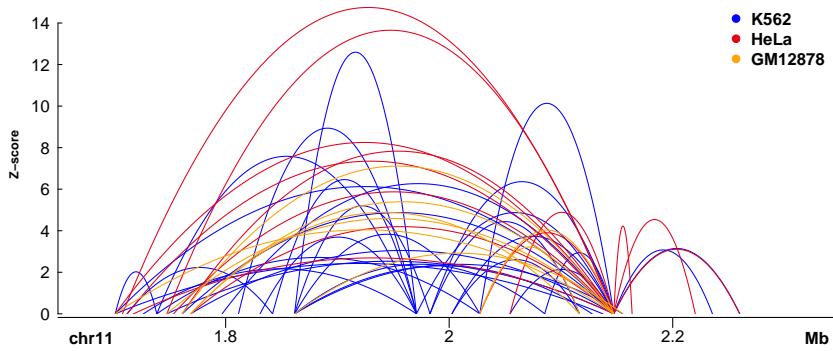
`plotBedpe()` can plot bedpe as arches. The height, linewidth, and color of each arch can be scaled to represent different aspects of the data. Here the height of the arches represents the Z-score of the 5C interaction, the color represents the cell line each interaction was detected in, and the line widths are kept constant (default lwd = 1).

```
> chrom           = "chr11"
> chromstart     = 1650000
> chromend       = 2350000
> pbpe = plotBedpe(Sushi_5C.bedpe,chrom,chromstart,chromend,
                     heights = Sushi_5C.bedpe$score,plottype="loops",
```

```

colorby=Sushi_5C.bedpe$samplenumber,
colorbycol=SushiColors(3))
> labelgenome(chrom, chromstart,chromend,n=3,scale="Mb")
> legend("topright",inset =0.01,legend=c("K562","HeLa","GM12878"),
  col=SushiColors(3)(3),pch=19,bty='n',text.font=2)
> axis(side=2,las=2,tcl=.2)
> mtext("Z-score",side=2,line=1.75,cex=.75,font=2)

```

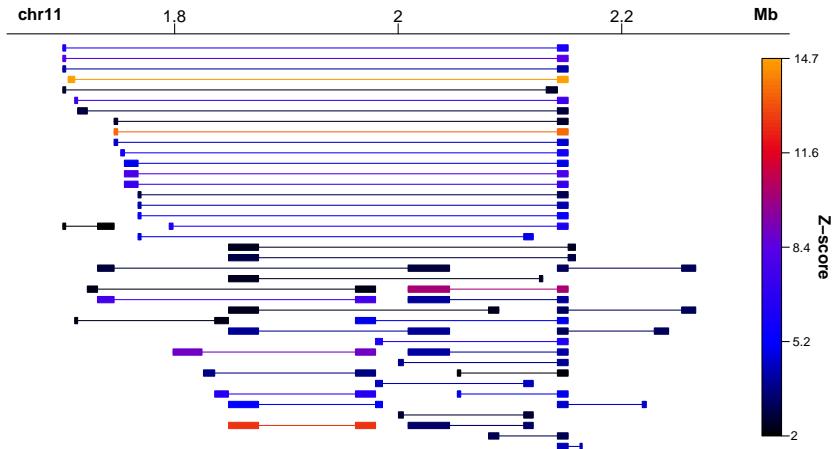


The plot can be flipped over the x-axis by setting `flip = TRUE`, Bedpe elements can be represented by boxes and straight lines by setting `plottype = "lines"`. And colors can be used to represent Z-scores by setting `colorby = "Sushi_5C.bedpe$score"`.

```

> chrom          = "chr11"
> chromstart     = 1650000
> chromend       = 2350000
> pbpe = plotBedpe(Sushi_5C.bedpe,chrom,chromstart,chromend,flip=TRUE,
  plottype="lines",colorby=Sushi_5C.bedpe$score,
  colorbycol=SushiColors(5))
> labelgenome(chrom, chromstart,chromend,side=3,n=3,scale="Mb")
> addlegend(pbpe[[1]],palette=pbpe[[2]],title="Z-score",side="right",bottominset=0.05,
  topinset=0.05,xoffset=-.035,labelside="right",width=0.025,title.offset=0.045)

```



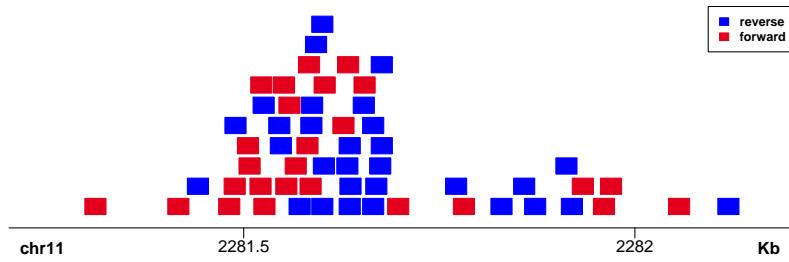
3.6 plotBed

`plotBed` provides multiple different ways to represent genomic data stored in bed format. Below are the first six lines of a bed file detailing reads from Pol2 ChIP-Seq analysis of K562 cells.

```
> head(Sushi_ChIPSeq_pol2.bed)
  chrom    start      end          name score strand
1 chr11 2280543 2280570 GGGCTCTCTCCGGCTTCCCTGTCCCGT    63   -1
2 chr11 2288946 2288973 CCTTCCCATCCGCAGGGCACCACATG  1000   -1
3 chr11 2272471 2272498 TGGGCATCAGTCAGGCTCCTCCCCAG  1000   -1
4 chr11 2288939 2288966 ATCCGCAGGGGCACCACATGAGTCACC 1000   -1
5 chr11 2281534 2281561 TGTCCCTAGTGACAAGTGGCCGGACTTG  250   -1
6 chr11 2286805 2286832 GGTGAGGCCAGCAGCTCCCTGGGGGG  250    1
```

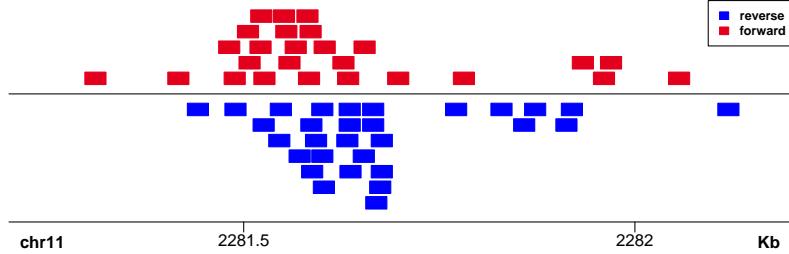
Leaving row set to `auto` provides a pile-sup style plot. Here the `colorby` argument is used to color the bed elements by the strand.

```
> chrom          = "chr11"
> chromstart     = 2281200
> chromend       = 2282200
> plotBed(beddata = Sushi_ChIPSeq_pol2.bed, chrom = chrom, chromstart = chromstart,
           chromend = chromend, colorby   = Sushi_ChIPSeq_pol2.bed$strand,
           colorbycol = SushiColors(2), row  = "auto", wiggle=0.001)
> labelgenome(chrom, chromstart, chromend, n=2, scale="Kb")
> legend("topright", inset=0, legend=c("reverse", "forward"), fill=SushiColors(2)(2),
         border=SushiColors(2)(2), text.font=2, cex=0.75)
```



Setting `splitstrand = TRUE` plots reads from different strands in two separate vertical regions.

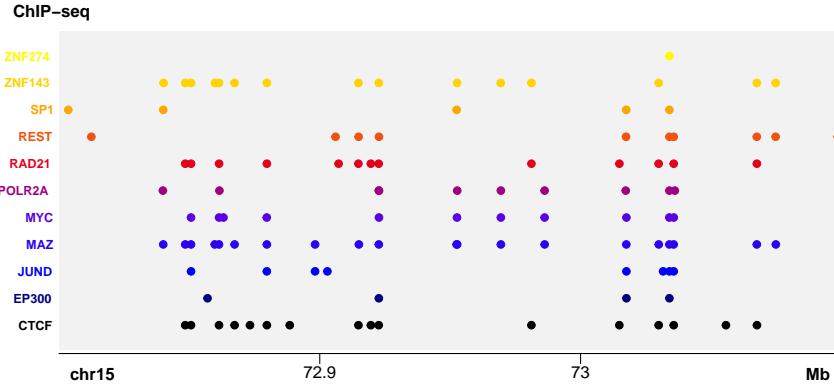
```
> chrom          = "chr11"
> chromstart    = 2281200
> chromend      = 2282200
> plotBed(beddata = Sushi_ChIPSeq_pol2.bed, chrom = chrom, chromstart = chromstart,
           chromend = chromend, colorby = Sushi_ChIPSeq_pol2.bed$strand,
           colorbycol = SushiColors(2), row = "auto", wiggle=0.001, splitstrand=TRUE)
> labelgenome(chrom, chromstart, chromend, n=2, scale="Kb")
> legend("topright", inset=0, legend=c("reverse", "forward"), fill=SushiColors(2)(2),
         border=SushiColors(2)(2), text.font=2, cex=0.75)
```



`plotBed` can also plot bed elements on different rows as specified by the user. First, we will use the Sushi function `maptocolors()` to assign a different color to each row.

```
> Sushi_ChIPSeq_severalfactors.bed$color =
  maptocolors(Sushi_ChIPSeq_severalfactors.bed$row,
  col=SushiColors(6))
```

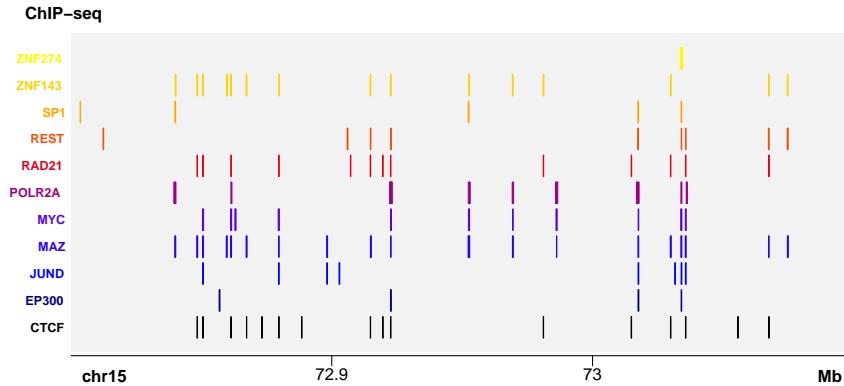
By providing row and color information `plotBed()` can be used to compare bed elements from different samples by plotting them on different rows.



```
> chrom          = "chr15"
> chromstart    = 72800000
> chromend      = 73100000
> plotBed(beddata = Sushi_ChIPSeq_severalfactors.bed,chrom = chrom,
           chromstart = chromstart,chromend =chromend,
           rownumber = Sushi_ChIPSeq_severalfactors.bed$row, type = "circles",
           color=Sushi_ChIPSeq_severalfactors.bed$color,row="given",
           plotbg="grey95",rowlabels=unique(Sushi_ChIPSeq_severalfactors.bed$name),
           rowlabelcol=unique(Sushi_ChIPSeq_severalfactors.bed$color),rowlabelcex=0.75)
> labelgenome(chrom,chromstart,chromend,n=3,scale="Mb")
> mtext("ChIP-seq",side=3, adj=-0.065,line=0.5,font=2)
```

That same data can be represented by rectangles that depict the actual width of each bed element.

```
> plotBed(beddata = Sushi_ChIPSeq_severalfactors.bed,chrom = chrom,
           chromstart = chromstart,chromend =chromend,
           rownumber = Sushi_ChIPSeq_severalfactors.bed$row, type = "region",
           color=Sushi_ChIPSeq_severalfactors.bed$color,row="given",
           plotbg="grey95",rowlabels=unique(Sushi_ChIPSeq_severalfactors.bed$name),
           rowlabelcol=unique(Sushi_ChIPSeq_severalfactors.bed$color),rowlabelcex=0.75)
> labelgenome(chrom,chromstart,chromend,n=3,scale="Mb")
> mtext("ChIP-seq",side=3, adj=-0.065,line=0.5,font=2)
```



`plotBed()` can also be used to plot heatmaps representing the density of bed elements. First, we will use the biomaRt function `getBM()` to get the gene information we require.

```
> chrom          = "chr15"
> chromstart    = 60000000
> chromend      = 80000000
> chrom_biomart = gsub("chr", "", chrom)
> mart=useMart(host='may2009.archive.ensembl.org', biomart='ENSEMBL_MART_ENSEMBL',
               dataset='hsapiens_gene_ensembl')
> geneinfobed = getBM(attributes = c("chromosome_name", "start_position", "end_position"),
                      filters= c("chromosome_name", "start", "end"),
                      values=list(chrom_biomart, chromstart, chromend), mart=mart)
> geneinfobed[,1] = paste("chr", geneinfobed[,1], sep="")
```

The data is in simple bed format with just three columns representing chromosome, start, and stop.

```
> head (geneinfobed)

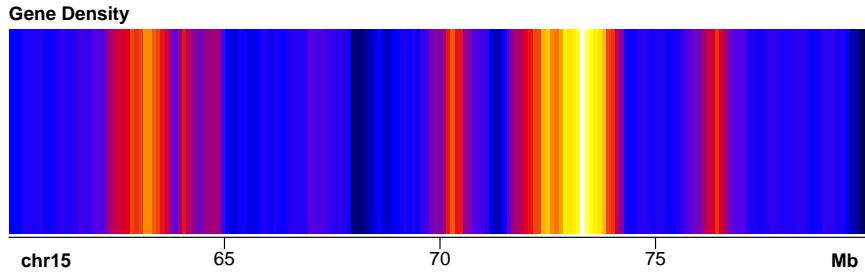
  chromosome_name start_position end_position
1         chr15        73372069     73372334
2         chr15        64580642     64580710
3         chr15        63375442     63375557
4         chr15        72570353     72570422
5         chr15        60903209     60903293
6         chr15        70130646     70130724
```

Now we can make a gene density plot using the `plotBed` function.

```

> plotBed(beddata = geneinfobed[!duplicated(geneinfobed),], chrom = chrom,
           chromstart = chromstart, chromend = chromend, row='supplied',
           palettes = list(SushiColors(7)), type = "density")
> labelgenome(chrom, chromstart, chromend, n=4, scale="Mb", edgeblankfraction=0.10)
> mtext("Gene Density", side=3, adj=0, line=0.20, font=2)

```



3.7 plotManhattan

`plotManhattan()` differs from most other Sushi functions in that it can plot multiple chromosomes in a single plot. Because of this `plotManhattan` requires some additional inputs. It requires an object in bed format describing the location of data points as well as vector of p-values (typically one of the columns of the bed file). But it also requires an genome object that describes which chromosomes to plot and their sizes (in bp). The genome object is very similar to the genome files used for bedtools.

The bed data should look something like this:

```

> head(Sushi_GWAS.bed)

  chr.hg18 pos.hg18 pos.hg18.1      rsid pval.GC.DBP V6
1   chr1    1695996    1695996  rs6603811  0.003110 .
2   chr1    1696020    1696020  rs7531583  0.000824 .
3   chr1    1698661    1698661  rs12044597  0.001280 .
4   chr1    1711339    1711339  rs2272908  0.001510 .
5   chr1    1712792    1712792  rs3737628  0.001490 .
6   chr1    1736016    1736016  rs12408690  0.004000 .

```

And the genome file should look like this:

```

> head(Sushi_hg18_genome)

  V1      V2
1 chr1  247249719
2 chr10 135374737

```

```

3 chr11 134452384
4 chr12 132349534
5 chr13 114142980
6 chr14 106368585

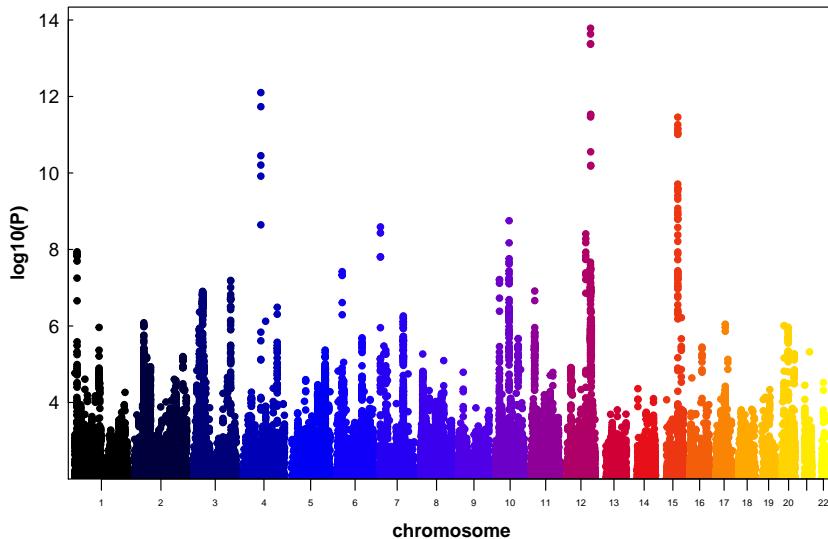
```

The `plotManhattan()` function is used to plot the data while the `labelgenome()` function is used to add the genome labels to the x-axis. The `labelgenome()` function also requires a genome object.

```

> plotManhattan(bedfile=Sushi_GWAS.bed,pvalues=Sushi_GWAS.bed[,5],
+                 col=SushiColors(6),genome=Sushi_hg18_genome,cex=0.75)
> labelgenome(genome=Sushi_hg18_genome,n=4,scale="Mb",
+               edgeblankfraction=0.20,cex.axis=.5)
> axis(side=2,las=2,tcl=.2)
> mtext("log10(P)",side=2,line=1.75,cex=1,font=2)
> mtext("chromosome",side=1,line=1.75,cex=1,font=2)

```



3.8 plotGenes

`plotGenes()` can be used to plot gene structures that are stored in bed format.

```

> head(Sushi_genes.bed)

  chrom      start      stop  gene score strand
1 chr15 73017309 73017438 COX5A      .     -1

```

```

2 chr15 72999672 72999836 COX5A      .      -1
3 chr15 73003042 73003164 COX5A      .      -1
4 chr15 73006160 73006281 COX5A      .      -1
5 chr15 73008510 73008626 COX5A      .      -1

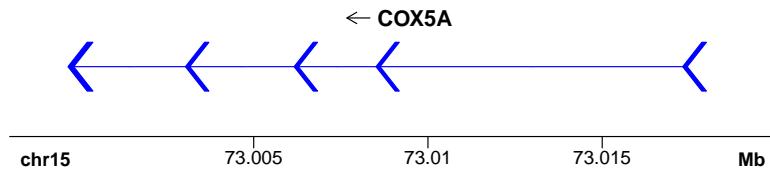
```

Using `plotGenes()` with arguments `bentline=FALSE` and `plotgenetype="arrow"` produces arrow and line gene structures.

```

> chrom          = "chr15"
> chromstart     = 72998000
> chromend       = 73020000
> pg = plotGenes(Sushi_genes.bed,chrom,chromstart,chromend ,
                  types=Sushi_genes.bed$type,maxrows=1,bheight=0.2,
                  plotgenetype="arrow",bentline=FALSE,
                  labeloffset=.4,fontsize=1.2,arrowlength = 0.025,
                  labeltext=TRUE)
> labelgenome( chrom, chromstart,chromend,n=3,scale="Mb")

```



This function can also be used to plot transcript structures. The first 20 lines of a data frame describing RNA seq data are shown below.

```

> Sushi_transcripts.bed[1:20,]

  chrom    start     stop      gene   score strand type
1 chr15 73062668 73062770 ENST00000362710 0.000000 -1 exon
2 chr15 73097788 73097929 ENST00000361900 0.000000  1 exon
3 chr15 73097264 73097365 ENST00000361900 0.000000  1 exon
4 chr15 73095987 73096143 ENST00000361900 0.000000  1 exon
5 chr15 73092071 73092199 ENST00000361900 0.000000  1 exon
6 chr15 73091234 73091240 ENST00000361900 0.000000  1 exon
7 chr15 73017309 73017408 ENST00000322347 31.488695 -1 exon
8 chr15 73006160 73006281 ENST00000322347 31.488695 -1 exon
9 chr15 73008510 73008626 ENST00000322347 31.488695 -1 exon
10 chr15 72984058 72984106 ENST00000357635 7.473977 -1 exon
11 chr15 72984548 72984625 ENST00000357635 7.473977 -1 exon
12 chr15 72985672 72985759 ENST00000357635 7.473977 -1 exon

```

```

13 chr15 72985981 72986194 ENST00000357635 7.473977 -1 exon
14 chr15 72975546 72975719 ENST00000379693 2.422616 1 exon
15 chr15 72972532 72972714 ENST00000379693 2.422616 1 exon
16 chr15 72972055 72972196 ENST00000379693 2.422616 1 exon
17 chr15 72970773 72970973 ENST00000379693 2.422616 1 exon
18 chr15 72969965 72970048 ENST00000379693 2.422616 1 exon
19 chr15 72972532 72972714 ENST00000352410 0.917141 1 exon
20 chr15 72972055 72972196 ENST00000352410 0.917141 1 exon

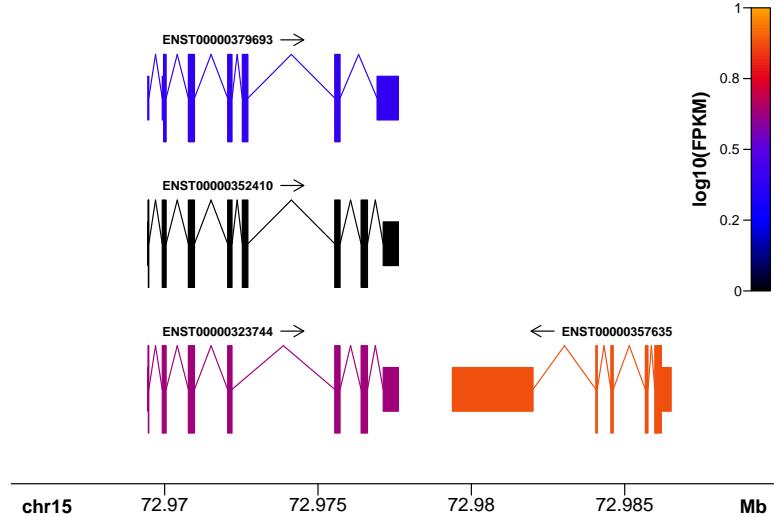
```

A vector type can be used to specify if each region is an 'exon' or 'utr' while `plotgenetype="box"` plots regions as a boxes rather than arrows. The data can be plotted using `plotGenes()`. The `colorby` argument is used to color the transcripts by $\log_{10}(\text{FPKM})$. UTR regions are drawn as shorter boxes than exons.

```

> chrom      = "chr15"
> chromstart = 72965000
> chromend   = 72990000
> pg = plotGenes(Sushi_transcripts.bed,chrom,chromstart,chromend ,
                  types = Sushi_transcripts.bed$type,
                  colorby=log10(Sushi_transcripts.bed$score+0.001),
                  colorbycol= SushiColors(5),colorbyrange=c(0,1.0),
                  labeltext=TRUE,maxrows=50,height=0.4,plotgenetype="box")
> labelgenome(chrom, chromstart,chromend,n=3,scale="Mb")
> addlegend(pg[[1]],palette=pg[[2]],title="log10(FPKM)",side="right",
            bottominset=0.4,topinset=0,xoffset=-.035,labelside="left",
            width=0.025,title.offset=0.055)

```



3.9 Zoom functions

A critical characteristic of the Sushi package is its ability to create highly customizable, publication-ready, multi-panel figures. Here, we will create a basic three panel figure and demonstrate how the zoom functions work (`zoomsregion` and `zoombox`). To illustrate these feature we will use the `plotBedgraph()` function to plot bedgrphah data representing a DNaseI hypersensitivity experiment in K562 cells.

In order to make a multipanel figure we will use the R function `layout`. Layout divides the device into rows and columns accoridng to a matrix you provide. The matrix also tells it which plots will appear on which parts of the plotting device. Below we make a 2 by 2 matrix. The entire top row will be used to plot the first plot while the bottom row with contain two plots. For more info on layout try `?layout`.

```
> layout(matrix(c(1,1,2,3),2, 2, byrow = TRUE))
> par(mar=c(3,4,1,1))
```

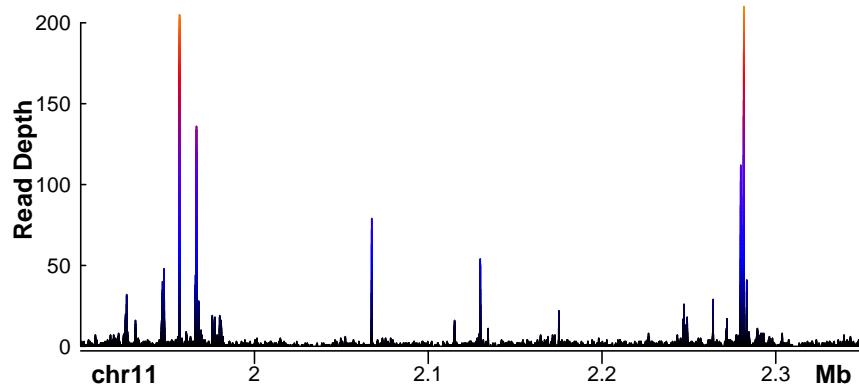
Next we will add the first plot

```
> chrom          = "chr11"
> chromstart     = 1900000
> chromend       = 2350000
```

```

> plotBedgraph(Sushi_DNaseI.bedgraph,chrom,chromstart=chromstart,
               chromend=chromend,colorbycol= SushiColors(5))
> labelgenome(chrom,chromstart=chromstart,chromend=chromend,n=4,
               scale="Mb")
> mtext("Read Depth",side=2,line=1.75,cex=1,font=2)
> axis(side=2,las=2,tcl=.2)

```

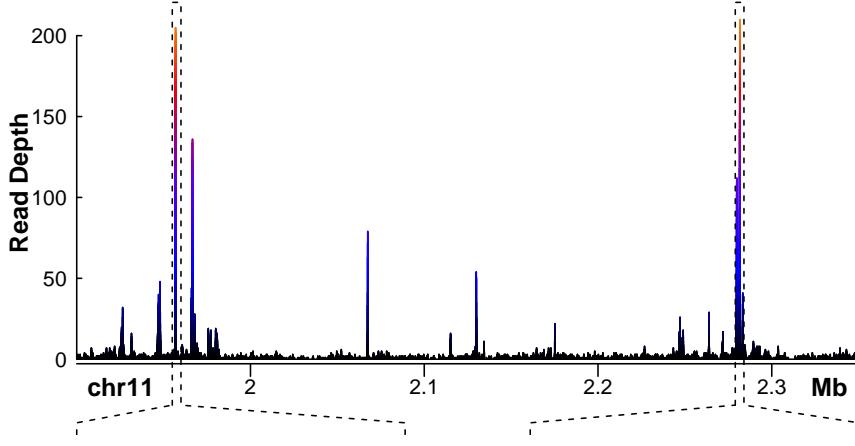


Next we will add the zoom regions using the function `zoomsregion()`. The argument `offsets` is used to precisely position the left and right edges of the widest part of the zoom.

```

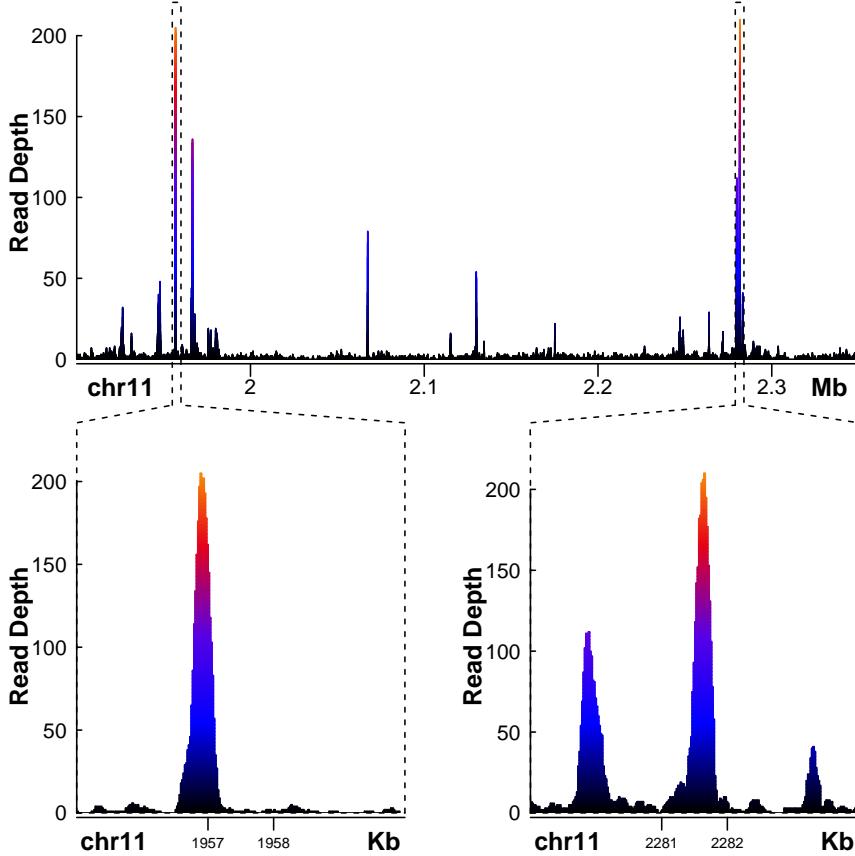
> zoomregion1      = c(1955000,1960000)
> zoomregion2      = c(2279000,2284000)
> zoomsregion(zoomregion1,extend=c(0.01,0.13),wideextend=0.05,
              offsets=c(0,0.580))
> zoomsregion(zoomregion2,extend=c(0.01,0.13),wideextend=0.05,
              offsets=c(0.580,0))

```



Then we can add each of the zoomed inset regions. For, each region we need execute the `zoombox` function in order to draw the lines around the new plots.

```
> plotBedgraph(Sushi_DNaseI.bedgraph,chrom,chromstart=zoomregion1[1],  
+               chromend=zoomregion1[2],colorbycol= SushiColors(5))  
> labelgenome(chrom,chromstart=zoomregion1[1],chromend=zoomregion1[2],  
+             n=4,scale="Kb",edgeblankfraction=0.2,cex.axis=.75)  
> zoombox()  
> mtext("Read Depth",side=2,line=1.75,cex=1,font=2)  
> axis(side=2,las=2,tcl=.2)  
> plotBedgraph(Sushi_DNaseI.bedgraph,chrom,chromstart=zoomregion2[1],  
+               chromend=zoomregion2[2],colorbycol= SushiColors(5))  
> labelgenome(chrom,chromstart=zoomregion2[1],chromend=zoomregion2[2],  
+             n=4,scale="Kb",edgeblankfraction=0.2,cex.axis=.75)  
> zoombox()  
> mtext("Read Depth",side=2,line=1.75,cex=1,font=2)  
> axis(side=2,las=2,tcl=.2)
```



3.10 Color functions

Sushi includes three functions to assist in the generating of R colors and color palettes: `SushiColors()`, `maptocolors()`, `opaque()`.

3.10.1 SushiColors

`SushiColors()` provides default color palettes for the Sushi package.

To see a list of available color palettes:

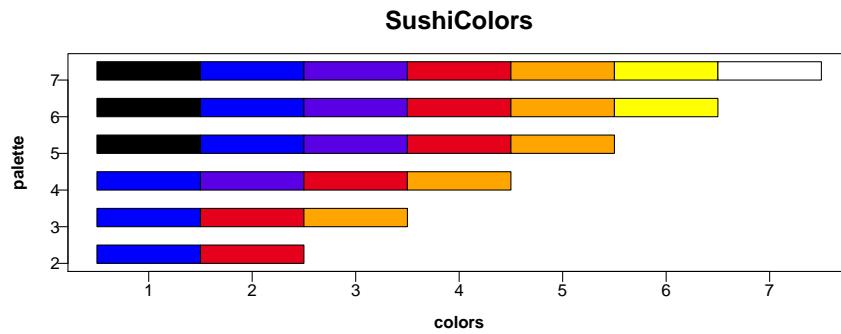
```
> SushiColors(palette='list')
[1] 2 3 4 5 6 7
```

To view the color palettes:

```

> plot(1,xlab='',xaxt='n',ylab='',yaxt='n',xlim=c(0.5,7.5),
      ylim=c(2,7.5),type='n')
> for (i in (2:7))
{
  for (j in (1:i))
  {
    rect(j-.5,i,j+.5,i+.5,col=SushiColors(i)(i)[j])
  }
}
> axis(side=2,at=(2:7),labels=(2:7),las=2)
> axis(side=1,at=(1:7),labels=(1:7))
> mtext("SushiColors",side=3,font=2, line=1, cex=1.5)
> mtext("colors",side=1,font=2, line=2)
> mtext("palette",side=2,font=2, line=2)

```



3.10.2 opaque

`opaque()` takes any color or vector of colors and makes them opaque. The degree of transparency is determined by the argument `transparency` which is a value between 0 and 1.

```

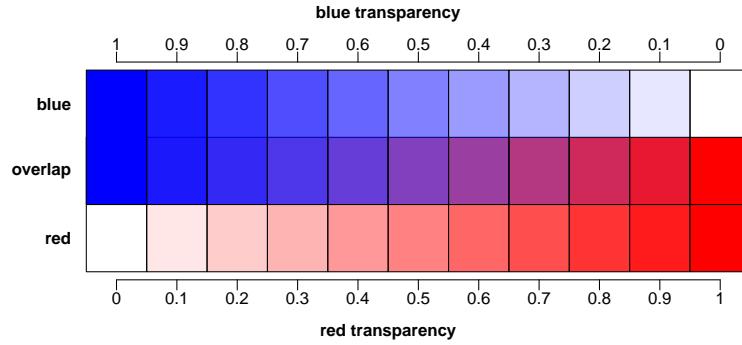
> plot(1,xlab='',xaxt='n',ylab='',yaxt='n',bty='n',type='n',
      xlim=c(-.15,1.05),ylim=c(-1,2))
> for (i in seq(0,1,by=0.1))
{
  rect(i-.05,-1,i+.05,1,col=opaque("red",transparency=i))
  rect(i-.05,0,i+.05,2,col=opaque("blue",transparency=1-i))
}
> axis(side=1,at=seq(0,1,by=0.1),labels=seq(0,1,by=0.1))
> mtext("red transparency",side=1,font=2, line=2)
> axis(side=3,at=seq(0,1,by=0.1),labels=seq(1,0,by=-0.1))
> mtext("blue transparency",side=3,font=2, line=2)
> text(-0.075,1.5,labels="blue",font=2,adj=1)

```

```

> text(-0.075,0.5,labels="overlap",font=2,adj=1)
> text(-0.075,-.5,labels="red",font=2,adj=1)

```



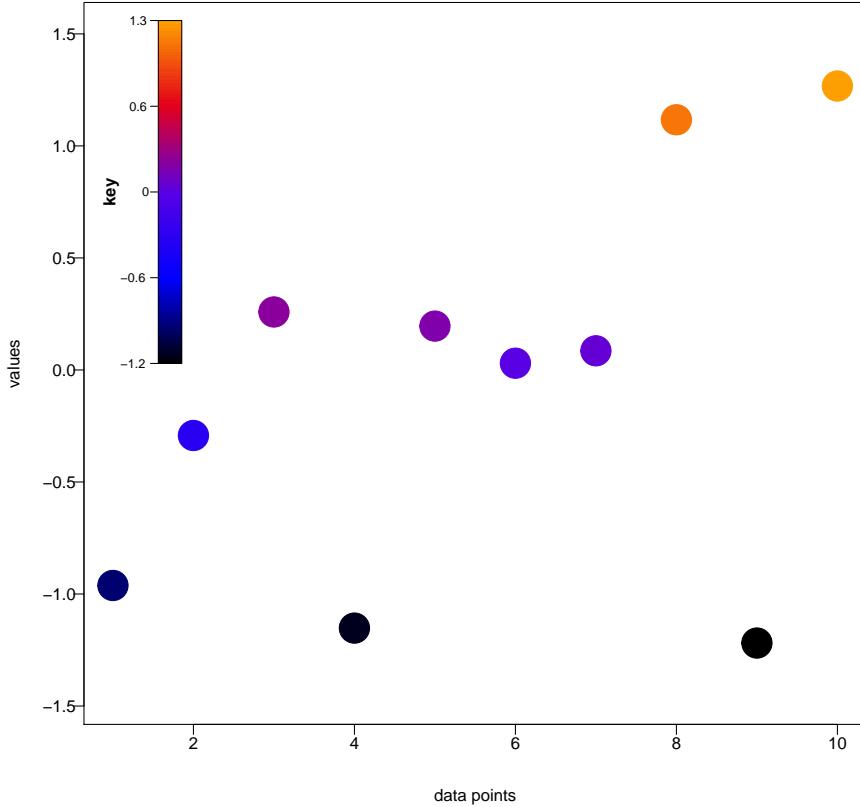
3.10.3 maptocolors

`maptocolors()` takes a vector of values and maps them to a color palette which can be used for plotting.

```

> set.seed(3)
> values = rnorm((1:10))
> colorpalette = SushiColors(5)
> plot(x=(1:10),y=values,col=maptocolors(values,colorpalette),
       pch=19,cex=4,xlab="data points",yaxt='n',ylim=range(values)*1.2)
> addlegend(range(values),title="key",palette=colorpalette,
            side='left',xoffset = -0.125,width=0.03,bottominset = 0.5, topinset = 0.025)
> axis(side=2,las=2)

```

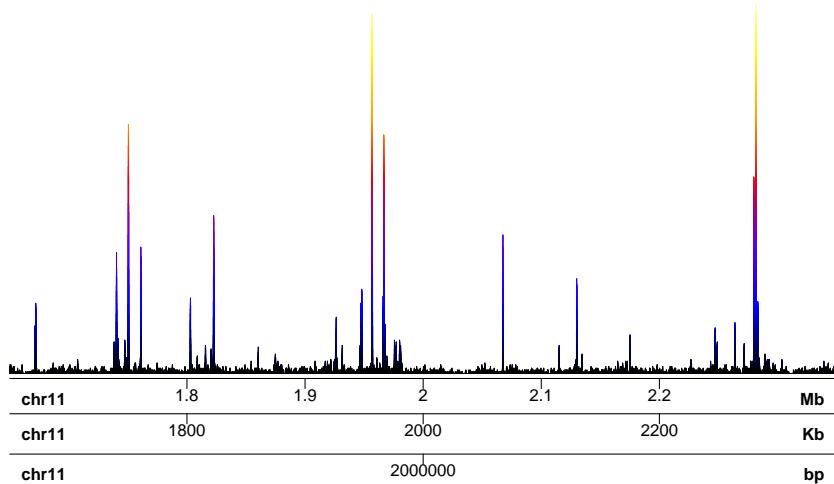


3.11 labeling functions

3.11.1 labelgenome

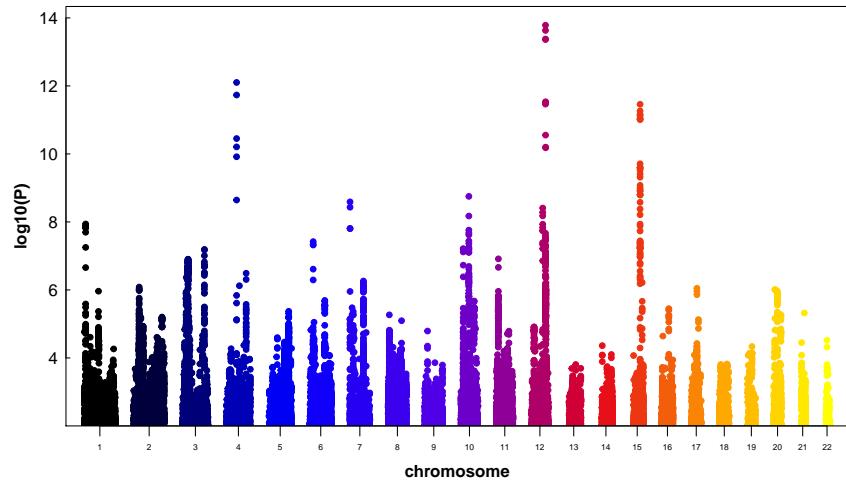
`labelgenome()` Add genome coordinates to the x-axis of a plot. The `line` argument can be used to offset the axis and `n` can be used to determine the desired number of tick marks.

```
> par(mar=c(8,3,3,1),mgp=c(3, .3, 0))
> plotBedgraph(Sushi_DNaseI.bedgraph,chrom="chr11",chromstart=1650000,
+                chromend=2350000,colorbycol=SushiColors(7))
> labelgenome(chrom="chr11",chromstart=1650000,chromend=2350000,
+              side=1,n=4,scale="Mb",line=.25)
> labelgenome(chrom="chr11",chromstart=1650000,chromend=2350000,
+              side=1,n=3,scale="Kb",line=2)
> labelgenome(chrom="chr11",chromstart=1650000,chromend=2350000,
+              side=1,n=1,scale="bp",line=4)
```



Manhattan plots include multiple genomes and labeling the axes of Manhattan plots requires the same `genome` object and value of `space` that were used to in `plotManhattan()`

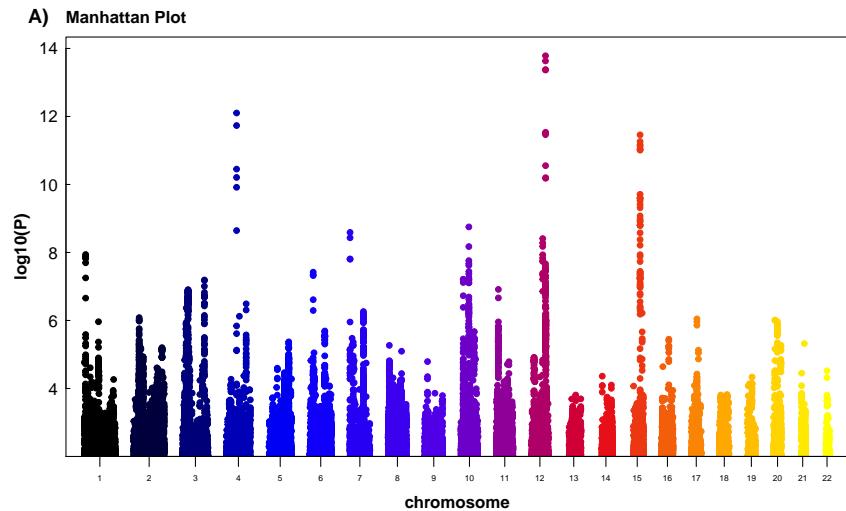
```
> plotManhattan(bedfile=Sushi_GWAS.bed,pvalues=Sushi_GWAS.bed[,5],
+                 col=SushiColors(6),genome=Sushi_hg18_genome,
+                 cex=0.75,space=0.05)
> labelgenome(genome=Sushi_hg18_genome,n=4,scale="Mb",
+              edgeblankfraction=0.20,cex.axis=.5,space=0.05)
> axis(side=2,las=2,tcl=.2)
> mtext("log10(P)",side=2,line=1.75,cex=1,font=2)
> mtext("chromosome",side=1,line=1.75,cex=1,font=2)
```



3.11.2 labelplot

Plot labels and titles can be added with the `labelplot()` function.

```
> labelplot("A", "Manhattan Plot")
```



References

- [1] Biomart. URL <http://www.biomart.org/>.
- [2] JR Dixon, S Selvaraj, F Yue, A Kim, Y Li, Y Shen, M Hu, JS Liu, and B Ren. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature*, 485, September 2012. doi: 10.1038/nature11082. PMID: 22495300.
- [3] International Consortium for Blood Pressure. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*, 478, September 2011. doi: 10.1038/nature10405. PMID: 21909115.
- [4] G Li, X Ruan, RK Auerbach, KS Sandhu, M Zheng, P Wang, HM Poh, Y Goh, J Lim, J Zhang, HS Sim, SQ Peh, FH Mulawadi, CT Ong, YL Orlov, S Hong, Z Zhang, S Landt, D Raha, G Euskirchen, CL Wei, W Ge, H Wang, C Davis, KI Fisher-Aylor, A Mortazavi, M Gerstein, T Gingeras, B Wold, Y Sun, MJ Fullwood, E Cheung, E Liu, WK Sung, M Snyder, and Y Ruan. Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. *Cell*, 148:84–98, January 2012. doi: 10.1016/j.cell.2011.12.014. PMID: 22265404.
- [5] S Neph, J Vierstra, AB Stergachis, AP Reynolds, E Haugen, B Vernot, RE Thurman, S John, R Sandstrom, AK Johnson, MT Maurano, R Humbert, E Rynes, H Wang, S Vong, K Lee, D Bates, M Diegel, V Roach, D Dunn, J Neri, A Schafer, RS Hansen, T Kutyavin, E Giste, M Weaver, T Canfield, P Sabo, M Zhang, G Balasundaram, R Byron, MJ MacCoss, JM Akey, MA Bender, M Groudine, R Kaul, and JA Stamatoyannopoulos. An expansive human regulatory lexicon encoded in transcription factor footprints. *Nature*, 489:83–90, September 2012. doi: 10.1038/nature11212. PMID: 22955618.
- [6] HS Rhee and BF Pugh. Comprehensive genome-wide protein-dna interactions detected at single-nucleotide resolution. *Cell*, 147, December 2011. doi: 10.1016/j.cell.2011.11.013. PMID: 22153082.
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- [8] The ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature*, 489(7414):57–74, September 2012. ISSN 1476-4687. doi: 10.1038/nature11247. URL <http://www.ncbi.nlm.nih.gov/pubmed/22955616>. PMID: 22955616.