

Segvis: A package for visualization of high throughput sequencing data along genomic segments

Rene Welch (welch@stat.wisc.edu) and Sündüz Keleş (keles@stat.wisc.edu)
Department of Statistics, University of Wisconsin - Madison
Madison, WI

April 2015

Contents

1 Overview	1
2 How to use Segvis?	2
2.1 Building a set of regions for <i>Segvis</i>	2
2.2 Creating a <i>segvis</i> object	3
2.3 Creating <i>segvis_block</i> object	4
3 Some examples	4
3.1 Plotting different marks accross specific peaks	4
3.2 Finding the summit of a specific mark's peaks	6
3.3 Summarizing the coverage for a set of same width regions	6
3.4 Subsetting and exploring the data with respect to user defined annotations	9
4 SessionInfo	12

1 Overview

This vignette provides an introduction to the visualization of sequencing data by using the *Segvis* package. The minimum input to the package includes:

1. Coordinates for regions of interest.
2. One or more bam files of aligned read data (e.g. from ChIP-seq experiments).

Segvis provides different tools to summarize and visualize these data, including but not limited to the following tasks:

- Extract read data of specified input regions.
- Plot data from different files (conditions) accross the same set of regions, e.g. peak plots for (SET or PET) ChIP-seq.
- Calculate and plot statistics(e.g. mean, median, variace, etc.) over a window around biologically meaningful coordinates (TSS, TFBS, etc.)
- Subset this regions according to user defined annotations.
- Plot the heatmap of signal curves accross regions separated by annotation.

2 How to use Segvis?

The package can be loaded with the command:

```
library(Segvis)
```

Different visualization of the data is done by the use of three following classes `segvis`, `segvis.block` and `segvis.block.list`. The first one is used to store the reads for a given bam file, the second is the one used to interact with the data and the third one is simply a list made exclusively of `segvis.block` objects.

2.1 Building a set of regions for Segvis

The minimum input for the package includes:

1. Coordinates for regions of interest.
2. One or more bam files of aligned read data (e.g. from ChIP-seq experiments).

The coordinates may be obtained by several means: Visual exploration in the genome browser, calling peaks from a ChIP-seq experiment, etc. To use *Segvis* it is necessary to load the regions of interest into a `segvis` object by formatting them as a *GRanges* object.

For example, if the peaks are saved in a **narrowPeak** file format¹, then we can load it into *R* by using:

```
peaks_file <- "../inst/extdata/peaks/encode_K562_Ctcf_peaks_first3chr.narrowPeak"
ctcf_peaks <- read.table(peaks_file)
head(ctcf_peaks,15)
```

##	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
## 1	chr1	114889057	114889538	.	0	.	671.4930	-1	4.57207	240
## 2	chr1	225662556	225663044	.	0	.	632.4595	-1	4.57207	249
## 3	chr1	150951878	150952345	.	0	.	601.5148	-1	4.57207	259
## 4	chr1	17036198	17036690	.	0	.	585.6260	-1	4.57207	255
## 5	chr1	35318207	35318710	.	0	.	520.4329	-1	4.57207	262
## 6	chr1	204776085	204776784	.	0	.	519.1454	-1	4.57207	376
## 7	chr1	33177715	33178175	.	0	.	514.7651	-1	4.57207	239
## 8	chr1	19239498	19239983	.	0	.	501.9843	-1	4.57207	230
## 9	chr1	38455665	38456081	.	0	.	496.4812	-1	4.57207	185
## 10	chr1	154989953	154990397	.	0	.	495.8747	-1	4.57207	221
## 11	chr1	9686983	9687432	.	0	.	489.9299	-1	4.57207	216
## 12	chr1	186344435	186344962	.	0	.	485.4386	-1	4.57207	239
## 13	chr1	26221873	26222297	.	0	.	481.1880	-1	4.57207	187
## 14	chr1	47902101	47902527	.	0	.	476.5053	-1	4.57207	185
## 15	chr1	109806165	109806687	.	0	.	476.2783	-1	4.57207	236

Then to convert it into a *GRanges* object we can use:

```
K <- 2000
ctcf_gr <- GRanges(seqnames = ctcf_peaks$V1,
  ranges = IRanges(start = ctcf_peaks$V2,
    end = ctcf_peaks$V3),strand = "*")
ctcf_gr <- ctcf_gr[order(ctcf_peaks$V7,decreasing=TRUE)[1:K]]
ctcf_gr
```

```
## GRanges object with 2000 ranges and 0 metadata columns:
##           seqnames           ranges strand
##           <Rle>             <IRanges>  <Rle>
```

¹A description of several common file formats is given in <https://genome.ucsc.edu/FAQ/FAQformat.html>

```
##      [1]      chr1 [114889057, 114889538]      *
##      [2]      chr1 [225662556, 225663044]      *
##      [3]      chr3 [195577700, 195578158]      *
##      [4]      chr1 [150951878, 150952345]      *
##      [5]      chr2 [ 91814978,  91815458]      *
##      ...      ...      ...      ...
## [1996]      chr1 [ 43823756,  43824034]      *
## [1997]      chr1 [205323464, 205323745]      *
## [1998]      chr2 [ 56410741,  56410988]      *
## [1999]      chr1 [152430955, 152431197]      *
## [2000]      chr1 [226033541, 226033846]      *
## -----
## seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

A complete description of the meaning of each column in the peaks file is given in <https://genome.ucsc.edu/FAQ/FAQformat.html#format12>. Using the signal values (on the 7th column), we are going to consider the top 2000 peaks.

2.2 Creating a segvis object

To create a *segvis* object, it is necessary to specify the following parameters:

- name - The name of the *segvis* object.
- regions - The regions to be loaded, in our case those are *ctcf_gr*.
- file - The file where the reads of the experiment are stored.
- maxBandwidth - The upper bound of all the possible bandwidths used to smooth the coverage plots when creating a *segvis_block* object.
- fragLen - The fragment length used to extend the fragment reads. If it is defined as zero, then it would use the original read widths.
- chr - The chromosomes for which the *segvis* object is defined. There are a couple of predefined cases as 'human' or 'mouse' to automatically consider all chromosomes in those genomes.
- isPET - A logical indicator if the reads of the experiment are paired-ended. In this case, the *fragLen* parameter is ignored.

```
ctcf <- buildSegvis(name = "ctcf_peaks",
  file = "../inst/extdata/reads/encode_K562_Ctcf_first3chr_Rep1.sort.bam",
  maxBandwidth = 101, fragLen = 200, isPET = FALSE,
  chr = c("chr1", "chr2", "chr3"))
regions(ctcf) <- ctcf_gr
ctcf
```

```
## Segvis for ctcf_peaks regions
## Paired-end Tags: FALSE
## Fragment length: 200
## Max Bandwidth: 101
## Using reads file:
## ../inst/extdata/reads/encode_K562_Ctcf_first3chr_Rep1.sort.bam
## Using regions for 3 chromosomes
## GRanges object with 2000 ranges and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>          <IRanges> <Rle>
##      [1]      chr1 [114889057, 114889538]      *
##      [2]      chr1 [225662556, 225663044]      *
##      [3]      chr1 [150951878, 150952345]      *
##      [4]      chr1 [ 17036198,  17036690]      *
```

```
##      [5]      chr1 [ 35318207,  35318710]      *
##      ...      ...      ...      ...
##      [1996]      chr3 [171171060, 171171364]      *
##      [1997]      chr3 [150863855, 150864128]      *
##      [1998]      chr3 [196756655, 196756932]      *
##      [1999]      chr3 [118603610, 118603955]      *
##      [2000]      chr3 [ 9768737,   9769156]      *
##      -----
##      seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

2.3 Creating segvis_block object

Segvis allows the use of several cores by using the parameter `mc`, which specifies the number of cores used by parallel processing. To create a `segvis_block` object it is necessary to follow a series of steps:

```
ctcf <- loadReads(ctcf, mc = 24)
ctcf <- matchReads(ctcf, mc = 24)
ctcf <- getCoverage(ctcf, mc = 24)
ctcf_block <- Segvis_block(ctcf, bw = 1, mc = 24)
```

To obtain the number of reads considered in an experiment:

```
ctcf_reads <- countReads(ctcf)
ctcf_reads
## [1] 6649370
```

In order to visually compare the enrichment level of more than one experiments it is necessary to normalize the experiment, which Segvis allows to do, by using:

```
normConst(ctcf_block) <- ctcf_reads
ctcf_block <- normalize(ctcf_block)
```

3 Some examples

3.1 Plotting different marks accross specific peaks

Using the same peaks as before. We are going to create a `segvis_block` for two additional marks, and plot all three marks the top 3 peaks, therefore we need to build two new `segvis_block` objects:

```
h3k27ac <- buildSegvis(name = "h3k27ac",
  file = "../inst/extdata/reads/encode_K562_H3k27ac_first3chr.sort.bam",
  maxBandwidth = 101, fragLen = 200, isPET = FALSE,
  chr = c("chr1", "chr2", "chr3"))
regions(h3k27ac) <- ctcf_gr

h3k27ac <- loadReads(h3k27ac, mc = 24)
h3k27ac <- matchReads(h3k27ac, mc = 24)
h3k27ac <- getCoverage(h3k27ac, mc = 24)
h3k27ac_block <- Segvis_block(h3k27ac, bw = 1, mc = 24)

h3k4me1 <- buildSegvis(name = "h3k4me1",
  file = "../inst/extdata/reads/encode_K562_H3k4me1_first3chr.sort.bam",
```

```

maxBandwidth = 101,fragLen = 200,isPET = FALSE,
chr = c("chr1","chr2","chr3"))
regions(h3k4me1) <- ctcf_gr

h3k4me1 <- loadReads(h3k4me1, mc = 24)
h3k4me1 <- matchReads(h3k4me1,mc = 24)
h3k4me1 <- getCoverage(h3k4me1,mc = 24)
h3k4me1_block <- Segvis_block(h3k4me1,bw = 1,mc = 24)

```

When sequencing data from different samples are considered together, they are often normalized to account for differences in the sequencing depths. The `normalize` function provides normalization functionalities. The default to to scale all the samples to 1M reads.

```

h3k27ac_reads <- countReads(h3k27ac)
normConst(h3k27ac_block) <- h3k27ac_reads
h3k27ac_block <- normalize(h3k27ac_block)

h3k4me1_reads <- countReads(h3k4me1)
normConst(h3k4me1_block) <- h3k4me1_reads
h3k4me1_block <- normalize(h3k4me1_block)

block_list <- Segvis_block_list(ctcf_block,h3k27ac_block,h3k4me1_block)
names(block_list) <- c("ctcf","h3k27ac","h3k4me1")

```

Then we can visualize all three marks over one regions by using the `plot_profiles` function:

```

rstart <- start(ctcf_gr)[1]
rend <- end(ctcf_gr)[1]
chr <- as.character(seqnames(ctcf_gr)[1])
iden <- function(x)x

p1 <- plot_profiles(block_list,condition = seqnames == chr & start == rstart,
  coord = rstart:rend,FUN = iden,mc=24)

```

The output of `plot_profiles` is a ggplot object, which we can modify it to obtains better looking plots.

```

p2 <- p1 + facet_grid(condition~.,scales = "free_y")
p3 <- p2 + scale_colour_brewer(palette = "Dark2")+theme(legend.position = "none")

```

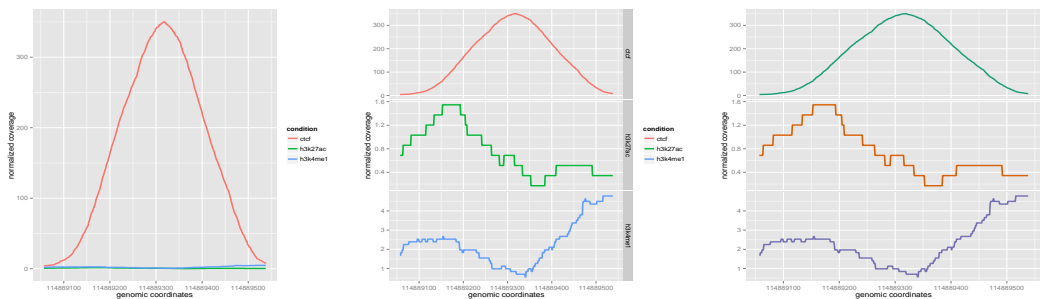


Figure 1: Initial use to plot the first Ctfc peak with all three marks (Ctfc, H3k27ac and H3k4me1). From left to right: p1, p2 and p3.

3.2 Finding the summit of a specific mark's peaks

One of the functions of *Segvis* is to calculate and plot statistics over a window around biologically meaningful coordinates like TSS, TFBS, etc. This coordinates are not always available, therefore *Segvis* allows to find the summits of peaks formed by a collection of genomic regions and fragment reads.

In subsection 2.3, we built the `ctcf` object which contains both regions and fragment reads, we can find the summit by using the `findSummit`, and then "add" it to the `segvis_block`'s regions:

```
summits <- findSummit(ctcf,bw=1,mc=24)
ctcf_block <- addColumn(ctcf_block,name="summit",col=summits)
ctcf_block

## Segvis profile: ctcf_peaks
## Bandwidth: 1
## The profile matrix IS scaled
## GRanges object with 2000 ranges and 1 metadata column:
##           seqnames           ranges strand | summit
##           <Rle>             <IRanges> <Rle> | <numeric>
##      [1]      chr1 [114889057, 114889538]   * | 114889318
##      [2]      chr1 [225662556, 225663044]   * | 225662819
##      [3]      chr1 [150951878, 150952345]   * | 150952129
##      [4]      chr1 [ 17036198,  17036690]   * |  17036475
##      [5]      chr1 [ 35318207,  35318710]   * |  35318418
##      ...      ...                ...      ...
## [1996]      chr3 [171171060, 171171364]   * | 171171197
## [1997]      chr3 [150863855, 150864128]   * | 150864001
## [1998]      chr3 [196756655, 196756932]   * | 196756778
## [1999]      chr3 [118603610, 118603955]   * | 118603783
## [2000]      chr3 [ 9768737,  9769156]     * |  9768843
## -----
## seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

3.3 Summarizing the coverage for a set of same width regions

Another functionality of *Segvis* is to calculate and plot statistics(e.g. mean, median, variace, etc.) over a window around biologically meaningful coordinates (TSS, TFBS, etc.), for which we are going to consider the summits that we found as those coordinates:

```
window_ext <- 500
new_start <- summits - window_ext
new_end <- summits + window_ext
new_regions <- GRanges(seqnames = seqnames(ctcf_gr),
  ranges = IRanges(start = new_start,end = new_end),strand = "*")
str(width(new_regions))

## int [1:2000] 1001 1001 1001 1001 1001 1001 1001 1001 1001 1001 ...

regions(ctcf) <- new_regions
regions(h3k27ac) <- new_regions
regions(h3k4me1) <- new_regions
all_segvis <- list("ctcf"=ctcf,"h3k27ac"=h3k27ac,"h3k4me1"=h3k4me1)
```

We are going to create a new `segvis_block.list` based on the new regions, for which we can apply the same methods `loadReads`, `matchReads`, `getCoverage` and `Segvis_block` to build it. This time, we are going to use two functions to apply all methods together:

```
do_all <- function(segvis_obj,bw,mc)
{
  segvis_obj <- loadReads(segvis_obj,mc = mc)
  segvis_obj <- matchReads(segvis_obj,mc = mc)
  segvis_obj <- getCoverage(segvis_obj,mc = mc)
  out <- Segvis_block(segvis_obj,bw = bw , mc = mc)
  return(out)
}

all_segvis_blocks <- lapply(all_segvis,do_all,bw = 1, mc = 24)
nreads <- c(ctcf_reads,h3k27ac_reads,h3k4me1_reads)

assign_and_normalize <- function(segvis_bl_obj,nreads)
{
  normConst(segvis_bl_obj) <- nreads
  segvis_bl_obj <- normalize(segvis_bl_obj)
  return(segvis_bl_obj)
}

all_segvis_blocks <- mapply(assign_and_normalize,
  all_segvis_blocks,nreads,SIMPLIFY=FALSE)
all_segvis_blocks <- Segvis_block_list(all_segvis_blocks)
names(all_segvis_blocks) <- names(all_segvis)
```

In this case, we have again 2000 regions with the same width of $m = 1001$, i.e. we can think it as a matrix of $K \times m$ and to analyze it we can apply the same function to all column vectors of this matrix:

```
q1 <- plot_profiles(all_segvis_blocks,FUN = mean,mc = 24,
  coord = -window_ext>window_ext)+xlab("distance to summit")+
  ylab("mean normalized counts")+
  scale_color_brewer(guide = guide_legend(title = "condition"),palette = "Dark2")+
  theme(legend.position = "top")+geom_vline(xintercept=0,linetype= 2)

q2 <- plot_profiles(all_segvis_blocks,FUN = median,mc = 24,
  coord = -window_ext>window_ext)+xlab("distance to summit")+
  ylab("median normalized counts")+
  scale_color_brewer(guide = guide_legend(title = "condition"),palette = "Dark2")+
  theme(legend.position = "top")+geom_vline(xintercept=0,linetype= 2)
```

We can even use functions created in the moment, the only need to take a vector argument and return a 1 - dimensional value.

```
varlog <- function(x)var(log(1 + x))

q3 <- plot_profiles(all_segvis_blocks,FUN = varlog,mc = 24,
  coord = -window_ext>window_ext)+xlab("distance to summit")+
  ylab("variance of log( 1 + normalized counts)")+
  scale_color_brewer(guide = guide_legend(title = "condition"),palette = "Dark2")+
  theme(legend.position = "top")+geom_vline(xintercept=0,linetype= 2)
```

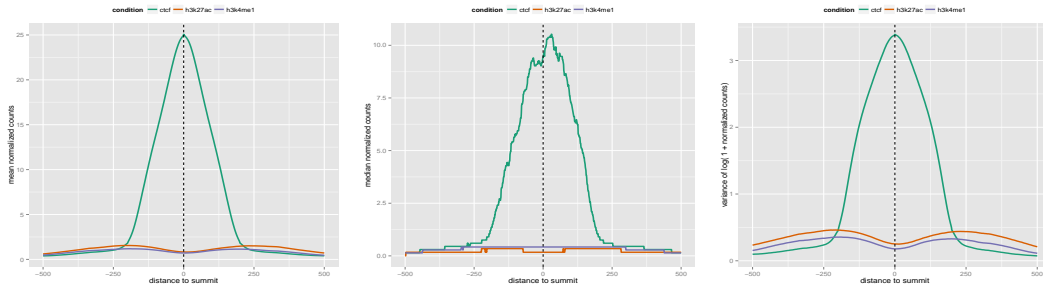


Figure 2: Mean, median and varlog profiles of all three marks (Ctfc,H3k27ac and H3k4me1) across the first 2000 Ctfc peaks

Another of Segvis functionalities is to calculate the data without generating the plot by the use of the `plot_data` function, which uses the same arguments as `plot_profiles`:

```
# x is the genomic coordinates or distance to summit in this case
# y is the normalized counts
# 1001 x 3 conditions = 3003 rows (in table below)
new_data1 <- plot_data(all_segvis_blocks,FUN = mean,trim = .1,mc = 24,
  coord = -window_ext:window_ext)
new_data2 <- plot_data(all_segvis_blocks,FUN = median,mc = 24,
  coord = -window_ext:window_ext)
new_data1
```

```
##      x      y condition
## 1: -500 0.2004889      ctfc
## 2: -499 0.2003009      ctfc
## 3: -498 0.2017108      ctfc
## 4: -497 0.2031207      ctfc
## 5: -496 0.2045307      ctfc
## ---
## 2999: 496 0.2799976 h3k4me1
## 3000: 497 0.2787677 h3k4me1
## 3001: 498 0.2769227 h3k4me1
## 3002: 499 0.2760441 h3k4me1
## 3003: 500 0.2755170 h3k4me1
```

And we can use this `data.table` into previously defined plot:

```
p4 <- p3 %>% new_data1 + ylab("average normalized counts")
p5 <- p3 %>% new_data2 + ylab("median normalized counts")
```

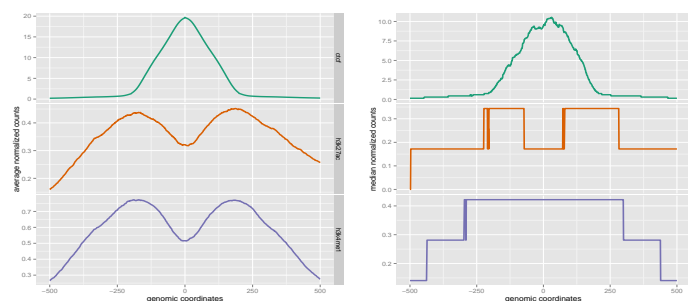


Figure 3: Mean and median coverage plot, by using a previously defined ggplot object

3.4 Subsetting and exploring the data with respect to user defined annotations

Segvis have methods to explore the data too. Lets consider as before that we want to explore the top 2000 Ctfc peaks, for which we already pre-processed a `segvis_block_list` made with Ctfc, H3k27ac and H3k4me1 fragment reads. Now, we want to explore this set of peaks respect to additional annotation information as to wheter this peaks overlap with Dnase accesible regions.

We read the Dnase hypersensitive sites from a file, and convert it to a `GRanges` object:

```
dnase_file <- "../inst/extdata/peaks/encode_K562_dnase_openChrom_first3chr.narrowPeak"
list.files("../inst/extdata/peaks/")

## [1] "encode_K562_Ctfc_peaks_first3chr.narrowPeak"
## [2] "encode_K562_dnase_openChrom_first3chr.narrowPeak"
## [3] "encode_K562_dnase_Uw_1_first3chr.narrowPeak"
## [4] "encode_K562_dnase_Uw_2_first3chr.narrowPeak"
## [5] "encode_K562_Pol2b_first3chr.narrowPeak"

dnase_sites = read.table(dnase_file)
dnase_gr <- GRanges(seqname = dnase_sites$V1,
  ranges = IRanges(start = dnase_sites$V2, end = dnase_sites$V3),
  strand = "*")
dnase_gr

## GRanges object with 29343 ranges and 0 metadata columns:
##           seqnames           ranges strand
##           <Rle>             <IRanges> <Rle>
##      [1]    chr1      [540885, 541038]    *
##      [2]    chr1      [713835, 714489]    *
##      [3]    chr1      [752734, 753083]    *
##      [4]    chr1      [762187, 763231]    *
##      [5]    chr1      [777812, 778569]    *
##      ...      ...                ...
## [29339]   chr3 [197686834, 197687293]    *
## [29340]   chr3 [197754035, 197754154]    *
## [29341]   chr3 [197759379, 197759678]    *
## [29342]   chr3 [197807020, 197808185]    *
## [29343]   chr3 [197834936, 197835028]    *
## -----
## seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

We count the number of overlaps between the Dnase hypersensitive sites and the top 2000 Ctfc peaks and add those columns to the `segvis_block_list` we calculated in :

```
nr_overlaps <- countOverlaps(regions(all_segvis_blocks[[1]]), dnase_gr)
all_segvis_blocks <- lapply(all_segvis_blocks,
  addColumn, name = "dnase_overlaps", col = nr_overlaps)
all_segvis_blocks[[1]]

## Segvis profile: ctfc_peaks
## Bandwidth: 1
## The profile matrix IS scaled
## GRanges object with 2000 ranges and 1 metadata column:
##           seqnames           ranges strand | dnase_overlaps
##           <Rle>             <IRanges> <Rle> | <integer>
##      [1]    chr1 [114888818, 114889818]    * |           1
##      [2]    chr1 [225662319, 225663319]    * |           1
##      [3]    chr1 [ 17035975,  17036975]    * |           1
```

```
##      [4]      chr1 [204776035, 204777035]      * |      1
##      [5]      chr1 [ 19239241,  19240241]      * |      1
##      ...      ...      ...      ...      ...
## [1996]      chr3 [193498236, 193499236]      * |      1
## [1997]      chr3 [ 11100887,  11101887]      * |      1
## [1998]      chr3 [126470748, 126471748]      * |      1
## [1999]      chr3 [ 52177993,  52178993]      * |      1
## [2000]      chr3 [ 10024309,  10025309]      * |      0
## -----
## seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

Then we can subset the data based on queries based on that annotation, for example:

```
ctcf_subset <- subset(all_segvis_blocks[[1]], dnase_overlaps > 0)
ctcf_subset

## Segvis profile: ctcf_peaks
## Bandwidth: 1
## The profile matrix IS scaled
## GRanges object with 1053 ranges and 1 metadata column:
##      seqnames      ranges strand | dnase_overlaps
##      <Rle>      <IRanges> <Rle> | <integer>
##      [1]      chr1 [114888818, 114889818]      * |      1
##      [2]      chr1 [225662319, 225663319]      * |      1
##      [3]      chr1 [ 17035975,  17036975]      * |      1
##      [4]      chr1 [204776035, 204777035]      * |      1
##      [5]      chr1 [ 19239241,  19240241]      * |      1
##      ...      ...      ...      ...      ...
## [1049]      chr3 [ 38537250,  38538250]      * |      1
## [1050]      chr3 [193498236, 193499236]      * |      1
## [1051]      chr3 [ 11100887,  11101887]      * |      1
## [1052]      chr3 [126470748, 126471748]      * |      1
## [1053]      chr3 [ 52177993,  52178993]      * |      1
## -----
## seqinfo: 3 sequences from an unspecified genome; no seqlengths

cover_table(ctcf_subset)

##      chr match      coord tagCounts
##      1: chr1      1 114888818 0.4511706
##      2: chr1      1 114888819 0.4511706
##      3: chr1      1 114888820 0.4511706
##      4: chr1      1 114888821 0.4511706
##      5: chr1      1 114888822 0.4511706
##      ---
## 1054049: chr3      343 52178989 0.1503902
## 1054050: chr3      343 52178990 0.1503902
## 1054051: chr3      343 52178991 0.1503902
## 1054052: chr3      343 52178992 0.1503902
## 1054053: chr3      343 52178993 0.1503902
```

Furthermore we can recreate the plots of figure 2 for the Ctfc peaks that overlap Dnse hypersensitive sites:

```
s1 <- plot_profiles(all_segvis_blocks, FUN = mean, mc = 24,
  condition = dnase_overlaps > 0 ,
  coord = -window_ext:window_ext)+xlab("distance to summit")+
  ylab("mean normalized counts")+
  theme_minimal()
```

```

scale_color_brewer(guide = guide_legend(title = "condition"),palette = "Dark2")+
theme(legend.position = "top")+geom_vline(xintercept=0,linetype= 2)

s2 <- plot_profiles(all_segvis_blocks,FUN = median,mc = 24,
  condition = dnase_overlaps > 0 ,
  coord = -window_ext:window_ext)+xlab("distance to summit")+
  ylab("median normalized counts")+
  scale_color_brewer(guide = guide_legend(title = "condition"),palette = "Dark2")+
  theme(legend.position = "top")+geom_vline(xintercept=0,linetype= 2)

s3 <- plot_profiles(all_segvis_blocks,FUN = varlog,mc = 24,
  condition = dnase_overlaps > 0 ,
  coord = -window_ext:window_ext)+xlab("distance to summit")+
  ylab("variance of log( 1 + normalized counts)")+
  scale_color_brewer(guide = guide_legend(title = "condition"),palette = "Dark2")+
  theme(legend.position = "top")+geom_vline(xintercept=0,linetype= 2)

```

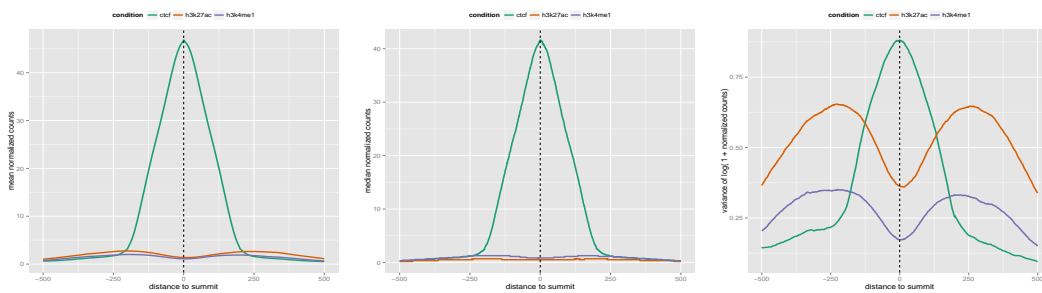


Figure 4: Mean, median and varlog profiles of all three marks (Ctf,H3k27ac and H3k4me1) across the first 2000 Ctf peaks that overlap with Dnse hypersensitive sites

Furthermore, we can use the `plot_data` method to build more complicated figures. For example:

```

mean_overlap_data <- plot_data(all_segvis_blocks,FUN = mean,mc = 24,
  condition = dnase_overlaps > 0,
  coord = -window_ext:window_ext)
mean_comp_data <- plot_data(all_segvis_blocks,FUN = mean,mc = 24,
  condition = dnase_overlaps == 0,
  coord = -window_ext:window_ext)
new_data <- rbind(mean_overlap_data[,overlap=="yes"],
  mean_comp_data[,overlap=="no"])
fancy_plot <- ggplot(new_data,aes(x,y,colour = condition))+geom_line(size=1.1)+
  facet_grid(overlap~.,scales = "free_y")+theme(legend.position = "top")+
  ggtitle("DHS overlaps")+geom_vline(xintercept = 0,linetype=2,size=1.1)+
  xlab("distance to summit")+ylab("average coverage")+
  scale_color_brewer(palette = "Set1")

```

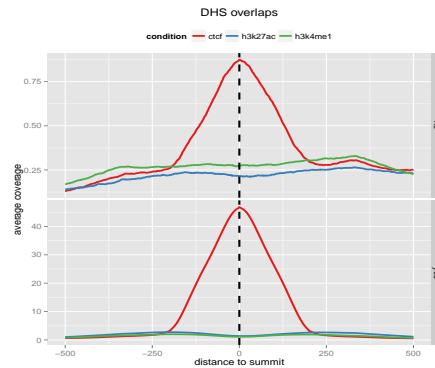


Figure 5: Average coverage of Ctf, H3k27ac and H3k4me1 over Ctf peaks overlapping with Dnase Hypersensitive Sites(DHS)

4 SessionInfo

```
toLatex(sessionInfo())
```

- R version 3.1.1 (2014-07-10), x86_64-redhat-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_US.UTF-8, LC_COLLATE=en_US.UTF-8, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C
- Base packages: base, datasets, graphics, grDevices, methods, parallel, stats, stats4, utils
- Other packages: BiocGenerics 0.12.1, Biostrings 2.34.1, data.table 1.9.4, GenomInfoDb 1.2.5, GenomicAlignments 1.2.2, GenomicRanges 1.18.4, ggplot2 1.0.1, IRanges 2.0.1, rBamtools 2.10.0, Rsamtools 1.18.3, S4Vectors 0.4.0, Segvis 2.0, XVector 0.6.0
- Loaded via a namespace (and not attached): base64enc 0.1-2, BatchJobs 1.6, BBmisc 1.9, BiocParallel 1.0.3, BiocStyle 1.4.1, bitops 1.0-6, brew 1.0-6, checkmate 1.5.2, chron 2.3-47, codetools 0.2-11, colorspace 1.2-6, DBI 0.3.1, digest 0.6.8, evaluate 0.7, fail 1.2, foreach 1.4.2, formatR 1.2, grid 3.1.1, gtable 0.1.2, highr 0.5, iterators 1.0.7, knitr 1.10.5, labeling 0.3, magrittr 1.5, MASS 7.3-39, munsell 0.4.2, plyr 1.8.2, proto 0.3-10, RColorBrewer 1.1-2, Rcpp 0.11.6, reshape2 1.4.1, RSQLite 1.0.0, scales 0.2.5, sendmailR 1.2-1, stringi 0.5-2, stringr 1.0.0, tools 3.1.1, zlibbioc 1.12.0