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

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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/example/encode_K562_Ctcf_peaks_first3chr.narrowPeak"</pre>
 ctcf_peaks <- read.table(peaks_file)</pre>
 head(ctcf_peaks,15)
##
       V1
                          V3 V4 V5 V6
                                            V7 V8
## 1
     chr1 33177715 33178175 . 0 . 514.7651 -1 4.57207 239
## 2 chr1 116681638 116682042 . 0 . 424.1570 -1 4.57207 216
## 3 chr1 41898407 41898778 . 0 . 375.1777 -1 4.57207 171
## 4 chr1 204716010 204716346 . 0 . 336.7304 -1 4.57207 151
## 5
    chr1 184951302 184951622 . 0 . 326.6078 -1 4.57207 165
## 6 chr1 26717999 26718312 . 0 . 308.8156 -1 4.57207 160
## 7 chr1 212838572 212838920 . 0 . 302.4028 -1 4.57207 176
## 8 chr1 44760418 44760726 . 0 . 237.7012 -1 4.57207 163
## 9 chr1 173883435 173883765 . 0 . 233.8862 -1 4.57207 171
## 10 chr1 110880945 110881308 . 0 . 222.4583 -1 4.57207 185
## 11 chr1 235578670 235578963 . 0 . 211.7700 -1 4.57207 151
## 12 chr1 206899686 206899983 . 0 . 203.5654 -1 4.57207 120
## 13 chr1 21985636 21985951 . 0
                                    . 199.1659 -1 4.57207 125
## 14 chr1 30579020 30579263 . 0 . 174.0020 -1 4.57207 138
## 15 chr1 110331751 110332065 . 0 . 155.2422 -1 4.57207 161
```

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

```
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)]
ctcf_gr

## GRanges object with 164 ranges and 0 metadata columns:
## seqnames ranges strand
## <Rle> <IRanges> <Rle>
## [1] chr1 [ 33177715, 33178175] *
```

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

```
##
       [2]
               chr1 [116681638, 116682042]
##
       [3]
               chr1 [ 41898407, 41898778]
               chr3 [ 15309468, 15309811]
       [4]
##
##
       [5]
               chr1 [204716010, 204716346]
##
       . . .
               . . .
               chr3 [ 9441505,
##
     [160]
                                 9441929]
##
     [161]
               chr2 [ 91808299, 91808723]
               chr1 [223397831, 223398255]
##
     [162]
##
     [163]
               chr3 [ 48315257, 48315681]
               chr3 [142443611, 142444035]
##
     [164]
##
     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 random 50 peaks per chromosome.

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 were 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",</pre>
    file = "../inst/extdata/example/encode_K562_Ctcf_first3chr_Rep1.sort.bam",
   maxBandwidth = 101, fragLen = 200, isPET = FALSE,
   chr = c("chr1","chr2","chr3"))
 regions(ctcf) <- ctcf_gr</pre>
  ctcf
## Segvis for ctcf_peaks regions
## Paired-end Tags: FALSE
## Fragment length: 200
## Max Bandwidth: 101
## Using reads file:
## ../inst/extdata/example/encode_K562_Ctcf_first3chr_Rep1.sort.bam
## Using regions for 3 chromosomes
## GRanges object with 164 ranges and 0 metadata columns:
           segnames
##
                                  ranges strand
##
              <Rle>
                                 <IRanges> <Rle>
               chr1 [ 33177715, 33178175]
       [1]
##
##
       [2]
             chr1 [116681638, 116682042]
       [3]
##
               chr1 [ 41898407, 41898778]
       [4]
              chr1 [204716010, 204716346]
##
```

```
##
       [5]
               chr1 [184951302, 184951622]
##
               chr3 [ 13460925, 13461349]
##
     [160]
##
     [161] chr3 [ 51424882, 51425306]
##
     [162]
             chr3 [ 9441505, 9441929]
               chr3 [ 48315257, 48315681]
##
     [163]
##
     [164]
              chr3 [142443611, 142444035]
##
##
    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)</pre>
```

To obtain the number of reads considered in an experiment:

```
ctcf_reads <- countReads(ctcf)
ctcf_reads
## [1] 22517</pre>
```

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)</pre>
```

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/example/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/example/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)</pre>
```

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:

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")</pre>
```

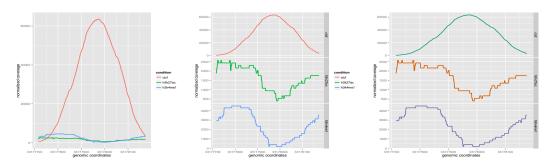


Figure 1: Initial use to plot the first Ctcf peak with all three marks (Ctcf, H3k27ac and H3k4me1). From left to right: p1, p2 and p3.

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)</pre>
  ctcf_block <- addColumn(ctcf_block,name="summit",col=summits)</pre>
  ctcf_block
## Segvis profile: ctcf_peaks
## Bandwidth: 1
## The profile matrix IS scaled
## GRanges object with 164 ranges and 1 metadata column:
                           ranges strand
##
           segnames
                                                              summit
               <Rle>
                                  <IRanges> <Rle>
##
                                                         | <numeric>
                                                        33177976
##
       [1]
              chr1 [ 33177715, 33178175]
       [2]
              chr1 [116681638, 116682042]
##
                                                        116681846
       [3] chr1 [ 41898407, 41898778]
[4] chr1 [204716010, 204716346]
[5] chr1 [184951302, 184951622]
##
                                                        41898584
##
                                                    *
                                                         204716175
##
                                                    *
                                                        184951465
##
       . . .
                . . .
     [160] chr3 [ 13460925, 13461349]
[161] chr3 [ 51424882, 51425306]
[162] chr3 [ 9441505, 9441929]
##
                                                  *
                                                            13461224
##
                                                            51425117
##
                                                    *
                                                             9441700
     [163] chr3 [ 48315257, 48315681]
##
                                                        48315297
            chr3 [142443611, 142444035]
##
     [164]
                                                         142443921
##
     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:

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)</pre>
  segvis_obj <- loadReads(segvis_obj,mc = mc)</pre>
  segvis_obj <- matchReads(segvis_obj,mc = mc)</pre>
  segvis_obj <- getCoverage(segvis_obj,mc = mc)</pre>
  out <- Segvis_block(segvis_obj,bw = bw , mc = mc)</pre>
  return(out)
all_segvis_blocks <- lapply(all_segvis,do_all,bw = 1, mc = 24)
nreads <- c(ctcf_reads,h3k27ac_reads,h3k4me1_reads)</pre>
assign_and_normalize <- function(segvis_bl_obj,nreads)</pre>
  normConst(segvis_bl_obj) <- nreads</pre>
  segvis_bl_obj <- normalize(segvis_bl_obj)</pre>
  return(segvis_bl_obj)
all_segvis_blocks <- mapply(assign_and_normalize,</pre>
  all_segvis_blocks,nreads,SIMPLIFY=FALSE)
all_segvis_blocks <- Segvis_block_list(all_segvis_blocks)</pre>
names(all_segvis_blocks) <- names(all_segvis)</pre>
```

In this case, we have again 50 regions for each chromosome with the same width of m=1001, i.e. we can think it as a matrix of $50 \times m$ and to analize 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)</pre>
```

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)</pre>
```

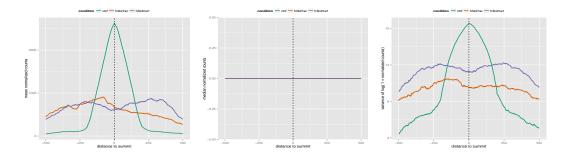


Figure 2: Mean, median and varlog profiles of all three marks (Ctcf,H3k27ac and H3k4me1) across random 150 Ctcf peaks

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 thise 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,</pre>
   coord = -window_ext:window_ext)
 new_data2 <- plot_data(all_segvis_blocks,FUN = median,mc = 24,</pre>
   coord = -window_ext:window_ext)
 new_data1
##
                      y condition
            X
##
      1: -500 15.14008
                             ctcf
##
      2: -499 15.14008
                             ctcf
##
      3: -498 15.14008
                             ctcf
##
      4: -497 15.14008
                             ctcf
      5: -496 15.14008
                             ctcf
##
## 2999:
         496 111.25550
                          h3k4me1
## 3000: 497 111.25550
                        h3k4me1
## 3001: 498 111.25550
                          h3k4me1
## 3002: 499 111.25550
                          h3k4me1
## 3003: 500 111.25550
                          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")
```

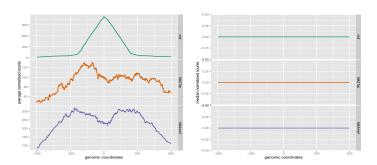


Figure 3: Mean and median coverage plot, by using a previously defined ggplot object

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 some Ctcf peaks, for which we already pre-processed a segvis_block_list made with Ctcf, 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 accessible regions.

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

```
dnase_file <- "../inst/extdata/example/encode_K562_dnase_openChrom_first3chr.narrowPeak"</pre>
  dnase_sites = read.table(dnase_file)
 dnase_gr <- GRanges(seqname = dnase_sites$V1,</pre>
    ranges = IRanges(start = dnase_sites$V2,end = dnase_sites$V3),
    strand = "*")
dnase_gr
## GRanges object with 109 ranges and 0 metadata columns:
##
           segnames
                                     ranges strand
              <Rle>
##
                                  <IRanges> <Rle>
##
       [1]
               chr1 [18648893, 18649322]
       [2]
##
               chr1 [21985597, 21986262]
       [3]
             chr1 [24894367, 24894616]
##
           chr1 [25286408, 25286558]
chr1 [25287078, 25287163]
##
       [4]
##
       [5]
##
       . . .
               . . .
             chr3 [163722135, 163722338]
##
     [105]
##
     [106]
               chr3 [172179354, 172179688]
##
     [107] chr3 [178864983, 178865248]
               chr3 [181413113, 181413660]
##
     [108]
##
     [109]
               chr3 [195617033, 195617148]
##
     seqinfo: 3 sequences from an unspecified genome; no seqlengths
##
```

We count the number of overlaps between the Dnase hypersensitive sites and the random 150 Ctcf peaks and add those columns to the segvis_block_list we calculated in :

```
nr_overlaps <- countOverlaps(regions(all_segvis_blocks[[1]]),dnase_gr)</pre>
 all_segvis_blocks <- lapply(all_segvis_blocks,</pre>
    addColumn,name = "dnase_overlaps",col = nr_overlaps)
  all_segvis_blocks[[1]]
## Segvis profile: ctcf_peaks
## Bandwidth: 1
## The profile matrix IS scaled
## GRanges object with 164 ranges and 1 metadata column:
##
           seqnames
                                    ranges strand | dnase_overlaps
##
              <Rle>
                                  <IRanges> <Rle>
                                                             <integer>
               chr1 [ 33177476, 33178476]
##
       [1]
                                                                      1
##
       [2]
               chr1 [116681346, 116682346]
                                                                      1
##
       [3]
               chr1 [ 41898084, 41899084]
                                                                      1
               chr1 [184950965, 184951965]
##
       [4]
                                                                      1
               chr1 [212838271, 212839271]
##
       [5]
                                                                      1
##
       . . .
                . . .
                                                                    . . .
               chr3 [125931556, 125932556]
##
     [160]
                                                                     1
##
     Г161]
               chr3 [ 4625852, 4626852]
                                                                     1
               chr3 [ 13460724, 13461724]
     [162]
                                                                      1
##
##
     [163] chr3 [ 48314797, 48315797]
```

```
## [164] chr3 [142443421, 142444421] * | 1
## -----
## seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

Then we can recreate the plots of figure 2 for the Ctcf 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")+
  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)
```

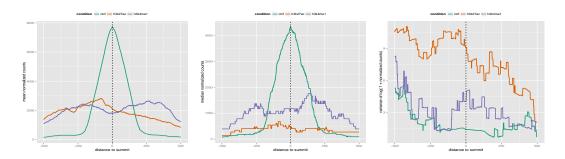


Figure 4: Mean, median and varlog profiles of all three marks (Ctcf,H3k27ac and H3k4me1) across the random Ctcf peaks that overlap with Dnse hypersentive sites

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")+</pre>
```

scale_color_brewer(palette = "Set1")

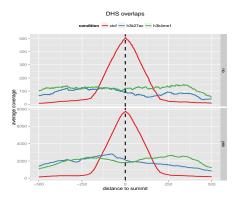


Figure 5: Average coverage of Ctcf, H3k27ac and H3k4me1 over Ctcf peaks overlapping with Dnase Hypersensitive Sites(DHS)

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, GenomeInfoDb 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