

genomation
package

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Usage and
ubiquity of
genomic
interval
summaries

Using
genomation

More
information

genomation

a toolkit to summarize, annotate and visualize genomic intervals

Altuna Akalin¹



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^{1*}* presented by. Package developed by Altuna Akalin and Vedran Franke



Quick introduction

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- The **genomation** is an **R** package that expedites genomic interval summary and annotation. It has the following features
 - ➊ Annotation of genomic intervals: e.g. see what % of your intervals overlap with exon/intron/promoters

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 - ① Annotation of genomic intervals: e.g. see what % of your intervals overlap with exon/intron/promoters
 - ② Summary of genomic scores or read coverages over pre-defined regions
 - e.g. extract the conservation profile over ChIP-seq binding sites (equi-width regions) or CpG islands (nonequi-width regions)

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 - ③ Visualize genomic interval summaries as meta-region plots or heatmaps.

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 - ③ Visualize genomic interval summaries as meta-region plots or heatmaps.
 - ④ Work with multiple file formats
 - e.g. BAM, BED, bigWig, GFF and generic tabular text files containing chromosome location information.

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 - e.g. BAM, BED, bigWig, GFF and generic tabular text files containing chromosome location information.
 - ⑤ do all these in **R** :)

Genomic interval summaries are widely used

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- Summaries of genomic intervals are one of the useful ways to communicate high-dimensional data
- Traditionally, regions of interest are picked and distribution of genomic intervals are summarized on those regions

Genomic interval summaries are widely used: Examples from literature

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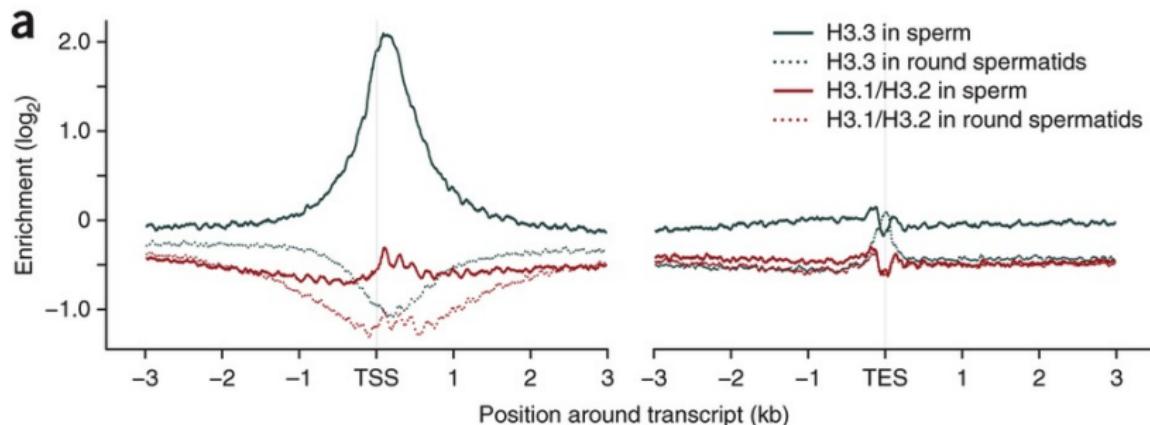


Figure : Erkek, S., et al. (2013). Molecular determinants of nucleosome retention at CpG-rich sequences in mouse spermatozoa. Nature Structural & Molecular Biology

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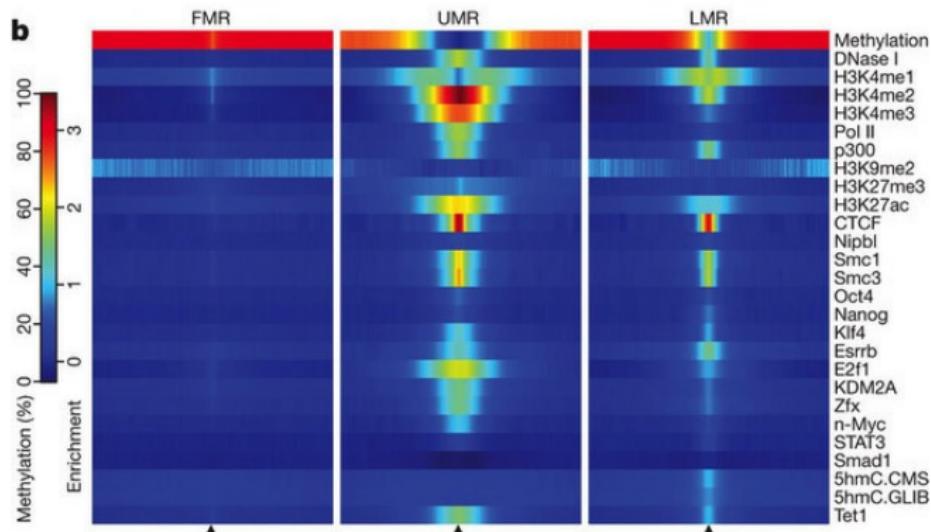


Figure : Stadler, M., Murr, R., Burger, L., et al. (2011). DNA-binding factors shape the mouse methylome at distal regulatory regions. Nature

Utility and futility of average profiles

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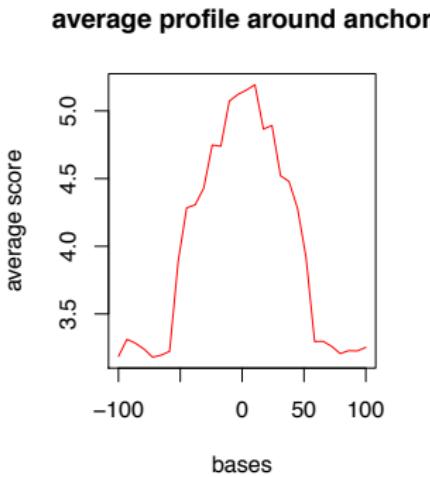
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Does this mean all of the windows (viewpoints) have a similar enrichment profile?



Utility and futility of average profiles

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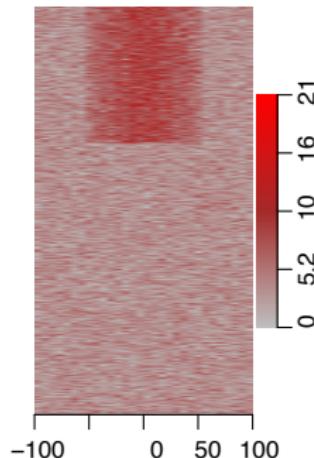
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Only 1/3 of windows have such enrichment. Be careful when you are interpreting the average profiles.



Genomic interval summaries are widely used: Examples from literature

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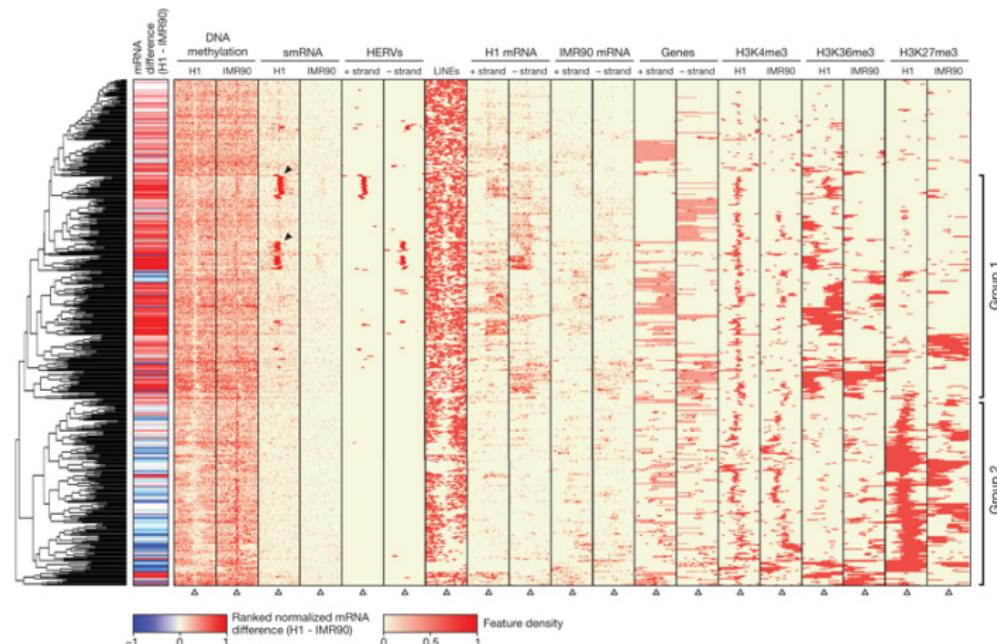


Figure : Lister, R., et al. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. Nature

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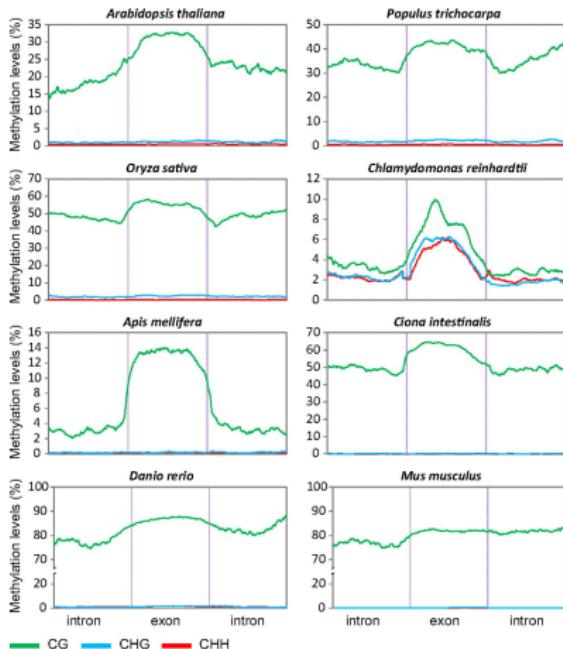


Figure : Feng, S. et al. (2010). Conservation and divergence of methylation patterning in plants and animals. PNAS

Issues to keep in mind when developing summary methods

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- Genomic data comes in many formats, we need a method that is able to **work with multiple flat file formats**
- We need a method that is not specialized on one type of data set such as read counts, it should also work on **other scoring schemes**(e.g. conservation scores) easily.
- Regions of interest are not always equi-width, you should be able to **normalize for length differences by binning**.
- **Multiple visualization options** and fast heatmap generation should be available
- **Clustering of regions** based on multiple summaries (e.g. binding for different TFs on the same set of regions) on the heatmap
- **Ease of use**, it should not take hours of coding to generate and visualize summaries.

Overview of genomation features

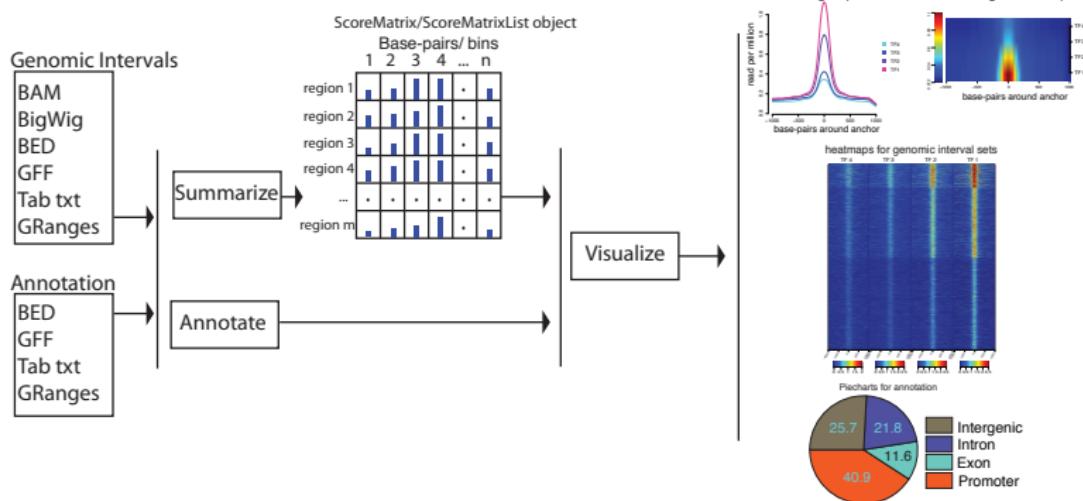
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installation of the package and the example data

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We can install the package and the data using *install_github()* function from the *devtools* package.

```
#install dependencies
install.packages( c("data.table","plyr","reshape2","ggplot2",
                     "gridBase", "devtools"))
source("http://bioconductor.org/biocLite.R")
biocLite(c("GenomicRanges", "rtracklayer", "impute", "Rsamtools"))

# install the packages
library(devtools)
install_github("genomation", username = "al2na")

# install the data package
# needed for examples
install_github("genomationData", username = "al2na")
```

Data import

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Various file formats can be used in genomation. You can read in annotation or your genomic intervals of interest.

```
library(genomation)
tab.file1 <- system.file("extdata/tab1.bed", package = "genomation")
readGeneric(tab.file1)

## GRanges with 6 ranges and 0 metadata columns:
##      seqnames      ranges strand
##      <Rle>      <IRanges>  <Rle>
## [1] chr21 [9437272, 9439473] *
## [2] chr21 [9483485, 9484663] *
## [3] chr21 [9647866, 9648116] *
## [4] chr21 [9708935, 9709231] *
## [5] chr21 [9825442, 9826296] *
## [6] chr21 [9909011, 9909218] *
## ---
## seqlengths:
##   chr21
##   NA
```

Extraction of data over pre-defined genomic regions

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ScoreMatrix() and **ScoreMatrixBin()** are functions used to extract data over predefined windows.

- **ScoreMatrix** is used when all of the windows have the same width (e.g. region around TSS)
- **ScoreMatrixBin** is designed for use with windows of unequal width (e.g. enrichment of methylation over exons).

```
data(cage)
data(promoters)
sm <- ScoreMatrix(target = cage, windows = promoters)
sm

## scoreMatrix with dims: 1055 2001
```

Visualizing ScoreMatrix: summary of genomic intervals over pre-defined regions

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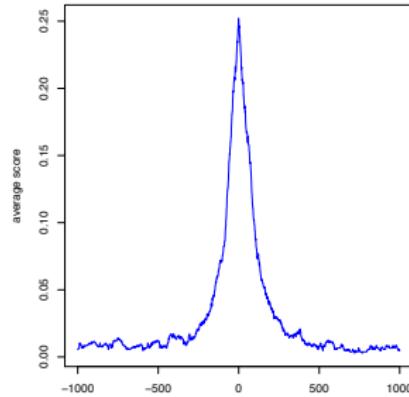
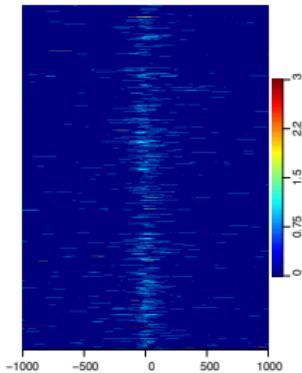
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plotMeta(), heatMeta(), heatMatrix() and multiHeatMatrix()
are the visualization functions.

```
oldmar <- par()$mar
par(oma = c(0, 0, 0, 0))
heatMatrix(sm, xcoords = c(-1000, 1000))
plotMeta(sm, xcoords = c(-1000, 1000), line.col="blue")
par(oma = oldmar)
```



Working with BAM files

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BAM files can also be used in ScoreMatrix() and ScoreMatrixBin() functions

```
bam.file = system.file('tests/test.bam', package='genomation')
windows = GRanges(rep(c(1,2),each=2),
                  IRanges(rep(c(1,2), times=2), width=5))
scores3 = ScoreMatrix(target=bam.file, windows=windows, type='bam')
```

Working with bigWig files

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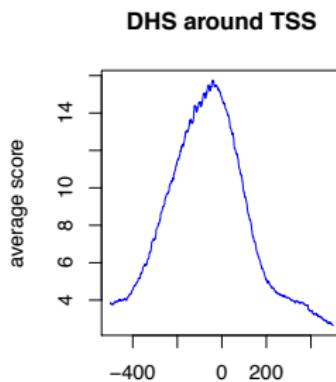
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ScoreMatrix() and **ScoreMatrixBin()** are functions can handle bigWig files. Here we use ENCODE DHS scores, downloaded from <http://goo.gl/fEVu0g>

```
my.bed12.file=system.file("extdata/chr21.refseq.hg19.bed",
                           package = "genomation")
feats=readTranscriptFeatures(my.bed12.file,up.flank=500,down.flank=500)
sm=ScoreMatrix(target="wgEncodeUwDnaseA549RawRep1.bw",
               windows=feats$promoters,type='bigWig',strand.aware=TRUE)
plotMeta(sm,xcoords=c(-500,500),main="DHS around TSS",line.col="blue")
```



Multiple profiles

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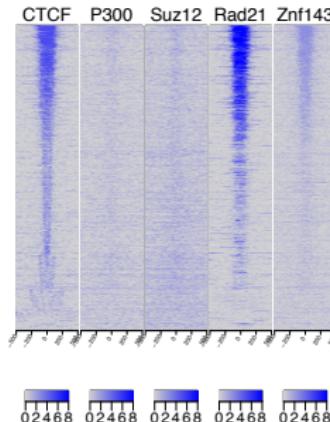
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Multiple heatmap profiles can be plotted using **multiHeatMatrix()** which takes in a **ScoreMatrixList** object. Here we used CTCF , P300 , Suz12 ,Rad21, Znf143 BAM files from genomationData package.

```
ctcf.peaks=readRDS("ctcf.peaks.rds")
dataPath = system.file("extdata", package = "genomationData")
bam.files = list.files(dataPath, full= T, pattern = "bam$")[c(1:4,6)]
smi = ScoreMatrixList(bam.files, ctcf.peaks, bin.num = 50, type = "bam")
names(smi)=c("CTCF","P300","Suz12","Rad21","Znf143")
multiHeatMatrix(sml, xcoords = c(-500, 500),cex.axis=0.35,common.scale = T,
                col = c("lightgray", "blue"),winsorize=c(0,95))
```



Multiple profiles

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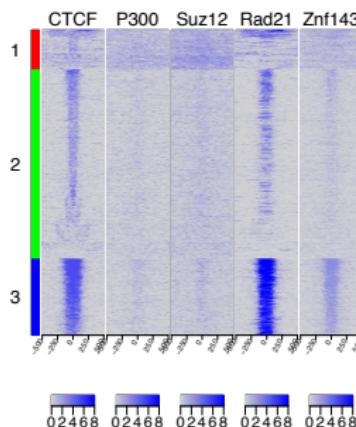
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multiHeatMatrix() can also apply K-means clustering. Extreme values are trimmed using with “winsorize” argument

```
multiHeatMatrix(sml, xcoords = c(-500, 500), kmeans=TRUE, k=3, common.scale = T,  
cex.axis=0.4, col = c("lightgray", "blue"), winsorize=c(0, 95))
```



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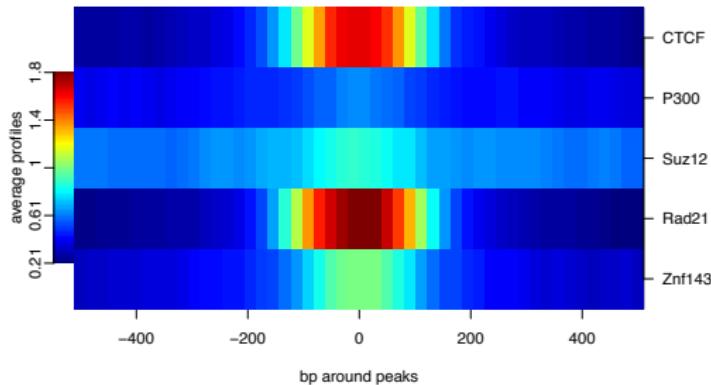
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Multiple average profiles can be visualized with **heatMeta()**. Here, we also apply a scaling function to all the matrices.

```
# take log2 of all matrices
sml2=scaleScoreMatrixList(sml,scalefun=function(x) log2(x+1))
heatMeta(sml2,legend.name="average profiles",xcoords=c(-500, 500),
         xlab="bp around peaks")
```



Multiple profiles

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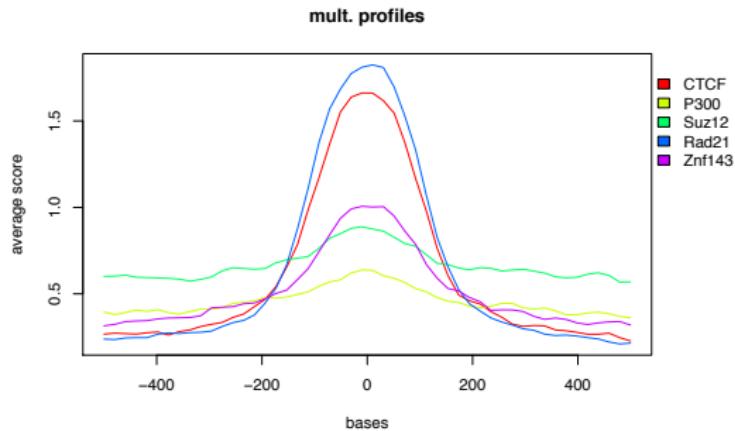
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Multiple average profiles can also be visualized with **plotMeta()**

```
plotMeta(sml2, profile.names=names(sml2),  
         xcoords=c(-500, 500),  
         main="mult. profiles")
```



Future work...

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- Explore overlap statistics between two genomic data sets: Does TF1 binding site locations overlap with TF2 sites more than expected?
- This is previously explored with GenometriCorr package. These functionality can be included in the form of a dependency.
- Performance improvement on certain functions, faster is always better...

Further information

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- The genomation package is available at <http://al2na.github.io/genomation>. You can find the link to the vignette on the webpage as well.
- Code that generated this presentation is available at http://github.com/al2na/genomation_presentation
- **Questions and bug reports**
 - You can view/open issues in github <https://github.com/al2na/genomation/issues?state=open>
 - You can ask questions by sending an e-mail to genomation@googlegroups.com or using the web interface to [google groups](#)
- Developed by Altuna Akalin and Vedran Franke

Session Info

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```
sessionInfo()

## R version 3.0.2 (2013-09-25)
## Platform: x86_64-apple-darwin10.8.0 (64-bit)
##
## locale:
## [1] C
##
## attached base packages:
## [1] methods   grid      stats      graphics  grDevices utils      datasets
## [8] base
##
## other attached packages:
## [1] genomation_0.99.0.2 knitr_1.5
##
## loaded via a namespace (and not attached):
## [1] BSgenome_1.30.0     BiocGenerics_0.8.0    Biostrings_2.30.0
## [4] GenomicRanges_1.14.3 IRanges_1.20.5      MASS_7.3-29
## [7] RColorBrewer_1.0-5   RCurl_1.95-4.1     Rsamtools_1.14.1
## [10] XML_3.95-0.2       XVector_0.2.0      bitops_1.0-6
## [13] colorspace_1.2-4    data.table_1.8.10   dichromat_2.0-0
## [16] digest_0.6.3       evaluate_0.5.1     formatR_0.10
## [19] ggplot2_0.9.3.1    gridBase_0.4-6     gtable_0.1.2
```

