

# Integrative Analysis of Epigenomic sequencing (and microarray) data with **Repitools**

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## 1 Introduction

**Repitools** is a package that allows exploratory as well as targeted statistical analysis of absolute and differential binding for ChIP-seq and MeDIP-seq data types, and gives visual summaries in a variety of formats. Some basic quality checking utilities are available for sequencing data. Much of the functionality available is implemented for both tiling microarrays and sequencing data, with very similar function calls for both types of data.

In this vignette, we highlight various features within the package. Further description of the package can be found in the associated Bioinformatics Applications Note<sup>1</sup> as well as in the help documents.

To start with, set a random seed, and load the **Repitools** package:

```
> options(prompt = " ", continue = " ")
  set.seed(4)
  library(Repitools)
```

## 2 Example Datasets

Caution : All data distributed with the package is a reduced version of the full dataset presented here. Also, all variable names have `.subset` appended to them. Therefore, the code presented here cannot simply be copied and pasted into **R**.

A small **GRangesList** of mapped short reads (four samples run on an Illumina Genome Analyser) is included with the package (for example, see `?binPlots`). This data has been published and is available here. LNCaP is a prostate cancer cell line, and PrEC is a (normal) prostate epithelial cell line. Here, the “IP” represents an MBD capture experiment, whereby a population of DNA fragments containing methylated DNA (generally in the CpG context) and “input” represents fragmented genomic DNA from the same cell lines.

Note that **GRanges** objects of mapped reads from many popular aligners can be created in **R** using the `readAligned` function in the **ShortRead** package, then coerced with `as(alnRdObj,`

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<sup>1</sup>Repitools: an R package for the analysis of enrichment-based epigenomic data

"GRanges"). Alternatively, two convenience methods `BAM2GRanges` and `BAM2GRangesList` in `Repitools` could also be used, if the reads were stored on disk in BAM format (this uses the `scanBam` function from the `Rsamtools` package). By default, these two methods read in only the uniquely-mapping reads. See the `ShortRead` package documentation for ideas about how to read other sequencing data into `GRanges` or `GRangesList` objects.

```
library(GenomicRanges)
data(samplesList)
class(samples.list)

[1] "GRangesList"
attr(,"package")
[1] "GenomicRanges"

names(samples.list)

[1] "PrEC input" "PrEC IP" "LNCaP input" "LNCaP IP"

elementNROWS(samples.list)

PrEC input      PrEC IP LNCaP input      LNCaP IP
 11061279      10008129      19119904      10139044
```

```
samples.list[[1]]
```

GRanges with 11061279 ranges and 0 elementMetadata values:

	seqnames	ranges	strand					
	<Rle>	<IRanges>	<Rle>					
[1]	chr1	[ 248, 283]	+					
[2]	chr1	[ 447, 482]	-					
[3]	chr1	[ 602, 637]	-					
[4]	chr1	[3182, 3217]	+					
[5]	chr1	[4783, 4818]	-					
[6]	chr1	[6287, 6322]	-					
[7]	chr1	[6310, 6345]	+					
[8]	chr1	[7340, 7375]	-					
[9]	chr1	[9103, 9138]	-					
...	...	...	...					
[11061271]	chrM	[16531, 16566]	+					
[11061272]	chrM	[16532, 16567]	+					
[11061273]	chrM	[16532, 16567]	-					
[11061274]	chrM	[16533, 16568]	-					
[11061275]	chrM	[16533, 16568]	+					
[11061276]	chrM	[16534, 16569]	-					
[11061277]	chrM	[16535, 16570]	-					
[11061278]	chrM	[16536, 16571]	+					
[11061279]	chrM	[16536, 16571]	-					
---								
seqlengths:								
	chr1	chr2	chr3	chr4	...	chrX	chrY	chrM
	247249719	242951149	199501827	191273063	...	154913754	57772954	16571

Also, an annotation of genes will be used. The annotation used here is based on one provided from Affymetrix for their Gene 1.0 ST expression arrays<sup>2</sup>. We will relate the epigenomic sequencing data to the Affymetrix gene expression measurements. Of course, users may wish to make use of the rich functionality available within the `GenomicFeatures` package.

```
gene.anno <- read.csv(system.file("data/geneAnno.csv", package = "Repitools"),
                      stringsAsFactors = FALSE)
```

```
head(gene.anno)
```

	name	chr	strand	start	end	symbol
1	7896759	chr1	+	781253	783614	LOC643837
2	7896761	chr1	+	850983	869824	SAMD11
3	7896779	chr1	+	885829	890958	KLHL17
4	7896798	chr1	+	891739	900345	PLEKHN1
5	7896817	chr1	+	938709	939782	ISG15
6	7896822	chr1	+	945365	981355	AGRN

```
dim(gene.anno)
```

```
[1] 24966      6
```

Lastly, there is matrix of gene expression changes, with each element related to the corresponding row in the gene annotation table. These values are moderated t-statistics (see the `limma` package) of background corrected and RMA normalised Affymetrix expression array experiments. The unprocessed array data is available here.

```
data(expr)
```

```
head(expr)
```

	t-stat
7896759	4.1130688
7896761	3.0691214
7896779	0.9724271
7896798	-0.5090460
7896817	2.1949896
7896822	-6.4049774

```
dim(expr)
```

```
[1] 24966      1
```

### 3 Quality Checking

As mentioned, two of the samples are MBD2 IPs, and two are inputs. Therefore, the IP samples should differ to the inputs in at least two ways. Firstly, they should be more CpG-rich, since we are enriching for methylated DNA, which rarely occurs outside of this sequence context. Secondly, DNA methylation tends to occur in peaks since CpG sites are often present in CpG-rich islands.

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<sup>2</sup>[http://www.affymetrix.com/Auth/analysis/downloads/na27/wtgene/HuGene-1\\_0-st-v1.na27.hg18.transcript.csv.zip](http://www.affymetrix.com/Auth/analysis/downloads/na27/wtgene/HuGene-1_0-st-v1.na27.hg18.transcript.csv.zip)

Conversely, the input samples should be distributed somewhat uniform genome-wide, aside from the usual mappability and GC content biases.

We can visualize the (log) frequencies of normalized coverage to get an idea of whether the reads occur in clusters or more dispersed, at least in a relative sense. For this, we can use `enrichmentPlot`. Similarly, we can calculate the CpG density of reads (or reads extended to a certain fragment size) and plot distributions across multiple samples using `cpgDensityPlot`, as below.

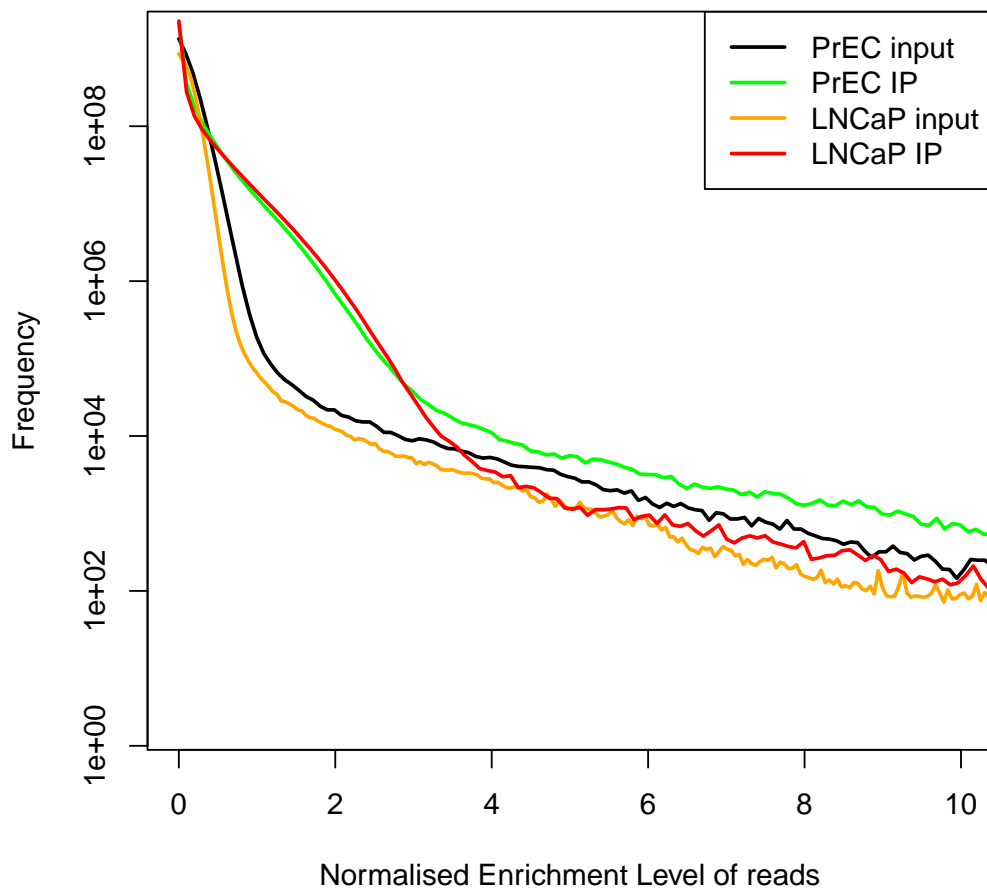
```
seqinfo(samples.list)
```

```
Seqinfo of length 25
```

seqnames	seqlengths	isCircular	genome
chr1	247249719	<NA>	hg18
chr2	242951149	<NA>	hg18
chr3	199501827	<NA>	hg18
chr4	191273063	<NA>	hg18
chr5	180857866	<NA>	hg18
chr6	170899992	<NA>	hg18
chr7	158821424	<NA>	hg18
chr8	146274826	<NA>	hg18
chr9	140273252	<NA>	hg18
...	...	...	...
chr17	78774742	<NA>	hg18
chr18	76117153	<NA>	hg18
chr19	63811651	<NA>	hg18
chr20	62435964	<NA>	hg18
chr21	46944323	<NA>	hg18
chr22	49691432	<NA>	hg18
chrX	154913754	<NA>	hg18
chrY	57772954	<NA>	hg18
chrM	16571	<NA>	hg18

```
enrichmentPlot(samples.list, seq.len = 300,  
                  cols = c("black", "green", "orange", "red"),  
                  xlim = c(0, 10), lwd = 2)
```

## Enrichment Plot

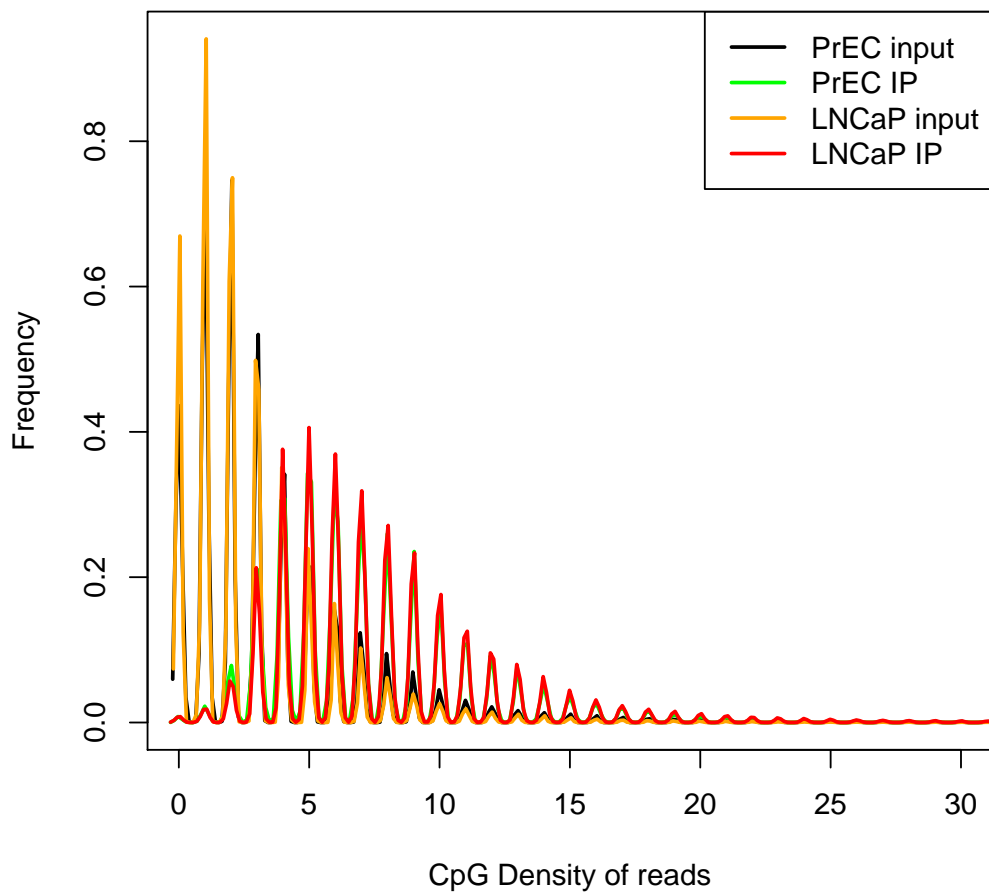


The code makes use of the `SeqInfo` annotation of `samples.list` to retrieve the maximum base of chromosomes. Normalization scales coverage value to “reads per 10 million”. The argument `seq.len=300` is passed in as the length to extend reads to, since that is approximately the real length of the fragments sequenced in this experiment. As expected, many more bases in the IP samples have high read coverages.

An alternative comparative visualization, which is somewhat specific to methylated DNA enrichment experiments, is a summary of the distribution of CpG density among reads/fragments:

```
library(BSgenome.Hsapiens.UCSC.hg18)
cpgDensityPlot(samples.list, organism = Hsapiens, w.function = "none", seq.len = 300,
               cols = c("black", "green", "orange", "red"), xlim = c(0, 30), lwd = 2)
```

## CpG Density Plot



The full genome sequence of the organism is required so that the (here, 300 base) DNA sequence can be fetched. In this example, the `BSgenome` package of the hg18 assembly for human is used (many other `BSgenome` objects for other organisms are available from Bioconductor). The `w.function` parameter allows the count of CpGs to be weighted. In this example, raw counts are used.

Notice that at lower CpG densities, the two input samples have a higher frequency of reads than the two IP samples. At higher CpG densities, this trend is reversed. This suggests that the enrichment of methylated CpGs has worked.

## 4 Analyses

### 4.1 Statistics of Differential Enrichment

The `blocksStats` function is a convenient way to do statistical tests of differential enrichment between two experimental conditions, using counts in regions of interest. The windows can be relative to some genomic landmarks, like transcription start sites (TSSs), and their size can be specified with the `up` and `down` parameters. If `up` and `down` are not provided, then windows are defined by start and end coordinates. The function leverages `edgeR`'s count modelling and its

adaptation of Fisher's exact test for assessing differential enrichment. The procedure also uses Bioconductor's facilities (i.e. `countOverlaps`) for counting mapped read in regions of the genome.

```
design.matrix <- matrix(c(0, -1, 0, 1), dimnames = list(names(samples.list), "C-N"))
design.matrix
```

```
      C-N
PrEC input    0
PrEC IP      -1
LNCaP input   0
LNCaP IP      1
```

```
stats <- blocksStats(samples.list, gene.anno, up = 2000, down = 0, seq.len = 300,
                      design = design.matrix)
```

Comparison of groups: 1 - -1

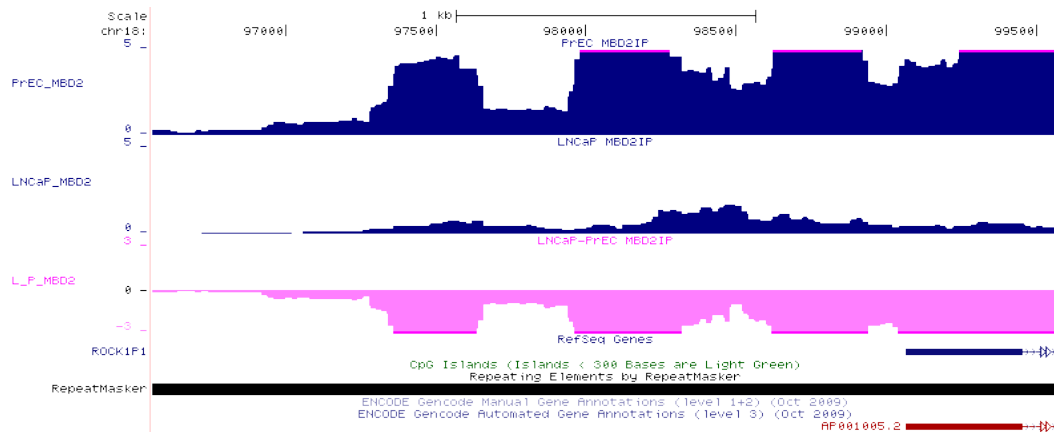
```
stats <- stats[order(stats$`adj.p.vals_C-N`), ]
head(stats)
```

	chr	start	end	width	strand	name	symbol	PrEC	input		
8019804	chr18	99064	112217	13154	+	8019804	ROCK1		600		
8015798	chr17	38802738	38821439	18702	-	8015798	---		87		
7904879	chr1	145017918	145018085	168	+	7904879	---		21		
7908529	chr1	196148257	196165896	17640	+	7908529	LHX9		16		
8115391	chr5	153834725	153838017	3293	-	8115391	HAND1		8		
7976848	chr14	100592279	100592359	81	+	7976848	---		29		
	PrEC	IP	LNCaP	input	LNCaP	IP	PrEC	IP_pseudo	LNCaP	IP_pseudo	logConc_C-N
8019804	397		686		58		399.588658		57.6237948		-16.01856
8015798	56		64		314		56.365618		311.9656941		-16.21306
7904879	13		28		153		13.085296		152.0084843		-17.78513
7908529	3		13		112		3.020114		111.2740395		-19.06788
8115391	4		28		95		4.026631		94.3841477		-18.97912
7976848	75		33		1		75.489482		0.9929602		-20.14963
	logFC_C-N	p.value_C-N	adj.p.vals_C-N								
8019804	-2.793764	1.662518e-64	4.150642e-60								
8015798	2.468516	6.449624e-44	8.051066e-40								
7904879	3.538199	1.681848e-31	1.399634e-27								
7908529	5.203643	1.220924e-29	7.620397e-26								
8115391	4.551106	1.238883e-23	6.185989e-20								
7976848	-6.247568	1.032321e-21	4.295489e-18								

Note that this is *not* a real design matrix (in a statistical sense), it is simply a way of specifying the two experiment conditions to compare (they must be 1 and -1).

The example above calculates statistics on regions that start 2000 bases upstream of the TSS and finish at the TSS, after the reads have been extended to being 300 bases. A coverage plot from UCSC browser illustrates the best found region. For the output table, the read counts are scaled as if there were 10 million reads covering the regions of interest.

Note that this procedure only works for simple 2-group comparisons. Using this strategy for more complicated designs requires manually creating the count tables (see `annotationCounts` below) and calling the GLM-based procedures (e.g. using real design matrices) within `edgeR`.



This differential enrichment strategy can be used on bins covering the entire genome. The `genomeBlocks` function can be used to generate windows along the genome.

## 4.2 Domains of Concordant Change

Another analysis of interest is the detection of *regions* where changes in expression (or an epigenetic mark, etc.) occur on a particular chromosome. The function `findClusters` addresses this need. The method of determining clusters requires a search through the column of scores (e.g. t-statistics) for a persistent change. Significance of clusters is determined by randomization. The order of the statistics is permuted a large number of times and the number of clusters found in the true statistics column and the permuted statistics columns is counted, ranging from a loose cutoff to a tight cutoff. A cutoff is chosen to control the user-specified FDR. Importantly, the table must be pre-sorted in positional order. This allows the user to use whatever definition of position they want. Note that the distance between features is not taken into account in this implementation.

```
stats.table <- data.frame(chr = "chr1", pos = 1:500, t.stat = rnorm(500))
stats.table[100:104, "t.stat"] <- rnorm(5, 5)
stats.table[200:204, "t.stat"] <- rnorm(5, -5)
stats.clustered <- findClusters(stats.table, score.col = 3, w.size = 5, n.med = 2, n.com)
cluster.1 <- which(stats.clustered$cluster == 1)
stats.clustered[cluster.1, ]
```

	chr	pos	t.stat	cluster
200	chr1	200	-5.7559870	1
201	chr1	201	-3.4111007	1
202	chr1	202	-4.7035741	1
203	chr1	203	-7.2695297	1
204	chr1	204	-4.8073155	1
205	chr1	205	0.3558196	1
206	chr1	206	-3.5198984	1
207	chr1	207	-1.1509771	1



In this example, a running window of 5 consecutive genes is calculated along each chromosome; the median value of those 5 genes is assigned to the middle gene. If, in the 5-gene window, there are at least 2 genes that have an assigned median above the cutoff being used (cutoffs of -2, -4, -6, -8, and -10 are tried), then those genes are candidate cluster-generating genes. Starting from a candidate gene, and working outwards until encountering a positive t-statistic, if a consecutive run of at least 3 genes with t-statistic being negative could be made, then this forms a cluster. The default estimated FDR of 0.05 is used.

### 4.3 Finding enriched regions in a single sample

Repitools contains an implementation of `ChromaBlocks` (described in Hawkins RD et al), designed to discover regions of the genome which are enriched for epigenetic marks, such as H3K27me3. Briefly, `ChromaBlocks` counts the number of sequencing reads aligned to adjacent bins in the genome in both Immunoprecipitated and Input samples, determines which bins exceed a cutoff for IP-Input enrichment (either specified or set at a supplied FDR by permutation) and returns regions of the genome where multiple adjacent bins are enriched.

Data from the Human Reference Epigenome Mapping Project is used to demonstrate `ChromaBlocks`. The data was downloaded from [here](#). Samples GSM466734, GSM466737, GSM466739, and GSM450270 are used here. The file containing the reads is available for download from the University of Zurich. After loading it into your R session, run the commands:

```
class(H1samples)

[1] "GRangesList"
attr(,"package")
[1] "GenomicRanges"

names(H1samples)

[1] "H3K4me1" "H3K27me3" "H3K36me3" "Input"

S4Vectors::elementNROWS(H1samples)

H3K4me1 H3K27me3 H3K36me3 Input
1201402 8673675 4151895 15289957

H3K27me3.blocks <- ChromaBlocks(rs.ip = H1samples["H3K27me3"],
                                rs.input = H1samples["Input"],
                                organism = Hsapiens, chrs = "chr20",
                                preset = "small", seq.len = 300)

Permutation 1.
Permutation 2.
Permutation 3.
Permutation 4.
Permutation 5.
Testing positive regions.
Using cutoff of 2.337163 for a FDR of 0.01
.
```

ChromaBlocks returns a ChromaResults object, from which an IRangesList of the regions determined to be enriched can be retrieved using the regions method.

```
H3K27me3.blocks
```

```
Object of class 'ChromaResults'.
```

```
1400 regions found with using a cutoff of 2.337163
```

```
regions(H3K27me3.blocks)
```

```
SimpleRangesList of length 1
```

```
$chr20
```

```
IRanges of length 1400
```

	start	end	width
[1]	60150	61550	1401
[2]	189750	191050	1301
[3]	228950	236350	7401
[4]	237050	243050	6001
[5]	243450	245550	2101
[6]	245850	248250	2401
[7]	259250	260950	1701
[8]	275950	277950	2001
[9]	279350	281050	1701
...	...	...	...
[1392]	62185050	62186750	1701
[1393]	62204850	62206750	1901
[1394]	62272950	62274750	1801
[1395]	62282050	62285250	3201
[1396]	62362950	62364450	1501
[1397]	62367750	62371150	3401
[1398]	62404450	62405650	1201
[1399]	62407650	62409450	1801
[1400]	62426850	62428550	1701

## 5 Visualisations

The visualisations in the following two subsections manipulate matrices of scores (such as coverages or intensities) in some way, such as clustering them, or summarising them by some defined grouping. The common interface for creating matrices of scores at regular distances from a reference point, such a TSS, is the featureScores function. The following example samples smoothed coverages between 5000 bases upstream of gene TSSs, and 1000 bases downstream of the TSS, at 1000 base intervals, in the PrEC and LNCaP immunoprecipitated samples. The scores are then subtracted from each other.

```
prostateCvgs <- featureScores(samples.list[c("PrEC IP", "LNCaP IP")], gene.anno,  
                               up = 5000, down = 1000, freq = 1000, s.width = 500)  
prostateCvgs
```

An object of class 'ScoresList'.

Tables: PrEC IP, LNCaP IP.

Features:

GRanges with 24966 ranges and 2 elementMetadata values:

	seqnames	ranges	strand		name	symbol
	<Rle>	<IRanges>	<Rle>		<integer>	<character>
[1]	chr1	[ 781253, 783614]	+		7896759	LOC643837
[2]	chr1	[ 850983, 869824]	+		7896761	SAMD11
[3]	chr1	[ 885829, 890958]	+		7896779	KLHL17
[4]	chr1	[ 891739, 900345]	+		7896798	PLEKHN1
[5]	chr1	[ 938709, 939782]	+		7896817	ISG15
[6]	chr1	[ 945365, 981355]	+		7896822	AGRN
[7]	chr1	[1092346, 1092441]	+		7896859	---
[8]	chr1	[1093105, 1093195]	+		7896861	---
[9]	chr1	[1094247, 1094330]	+		7896863	---
...	...	...	...	...	...	...
[24958]	chrY	[19611913, 19614093]	-		8177222	CD24
[24959]	chrY	[19640262, 19640361]	-		8177227	---
[24960]	chrY	[20076704, 20124427]	-		8177229	BCORL2
[24961]	chrY	[20326698, 20366197]	-		8177232	JARID1D
[24962]	chrY	[21036941, 21090502]	-		8177261	TTY10
[24963]	chrY	[21954227, 21957634]	-		8177273	---
[24964]	chrY	[22001970, 22008519]	-		8177280	---
[24965]	chrY	[22154873, 22165940]	-		8177282	TTY13
[24966]	chrY	[22852332, 22854411]	-		8177344	TTY5

---

seqlengths:

chr1	chr10	chr11	chr12	chr13	chr14	...	chr6	chr7	chr8	chr9	chrX	chrY
NA	NA	NA	NA	NA	NA	...	NA	NA	NA	NA	NA	NA

Region: 5000 bases up to 1000 bases down.

Smoothing: 500, 500 bases.

Sampling : 1000 bases.

```
prostateCvgs@scores <- list(tables(prostateCvgs)[[2]] - tables(prostateCvgs)[[1]])
names(prostateCvgs) <- "LNCaP - PrEC"
```

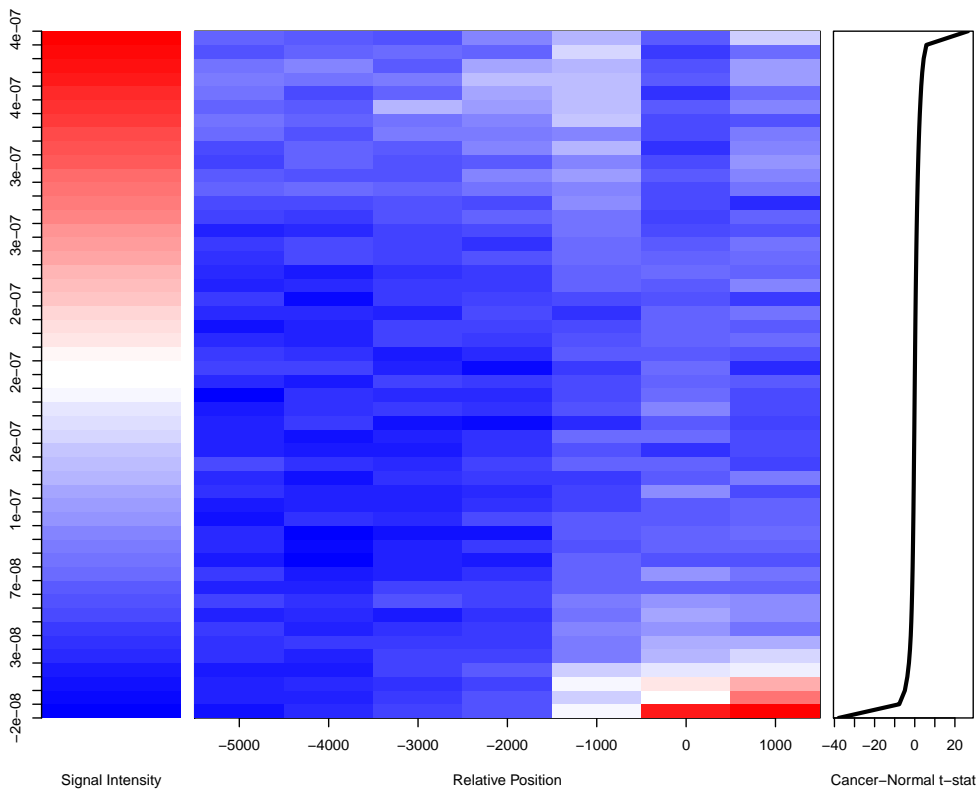
This object will be used in the next few visualisation functions.

## 5.1 Integrative analysis of epigenetics and gene expression

Epigenomic data is often gathered with other data, such as gene expression. It may be of interest to see the profile of epigenetic enrichment at a variety of distances from TSSs, stratified by gene expression level. The `binPlots` function is a convenient way to look at these interactions.

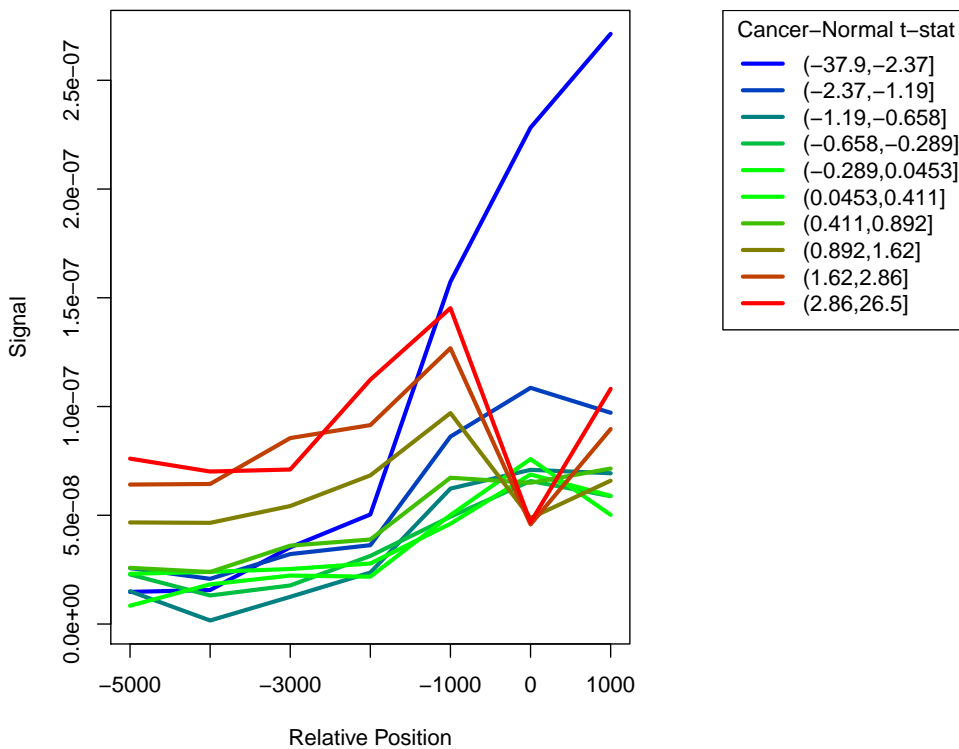
```
binPlots(prostateCvgs, ordering = expr, ord.label = "Cancer-Normal t-stat",
         plot.type = "heatmap", n.bins = 50)
```

Signal: LNCaP – PrEC Order: t-stat



Enrichment levels (here, differential enrichment) are split into bins based on the moderated t-statistics for change in expression. Signal for (differential) enrichment is averaged over genes in the bin and plotted as a heatmap. As expected, the genes that are silenced in cancer exhibit higher levels of DNA methylation around their TSS, compared to normal cells. This visualization can be represented as a lineplot, by setting `plot.type= "line"` (see below).

```
binPlots(prostateCvgs, ordering = expr, ord.label = "Cancer-Normal t-stat",  
         plot.type = "line", n.bins = 10)
```



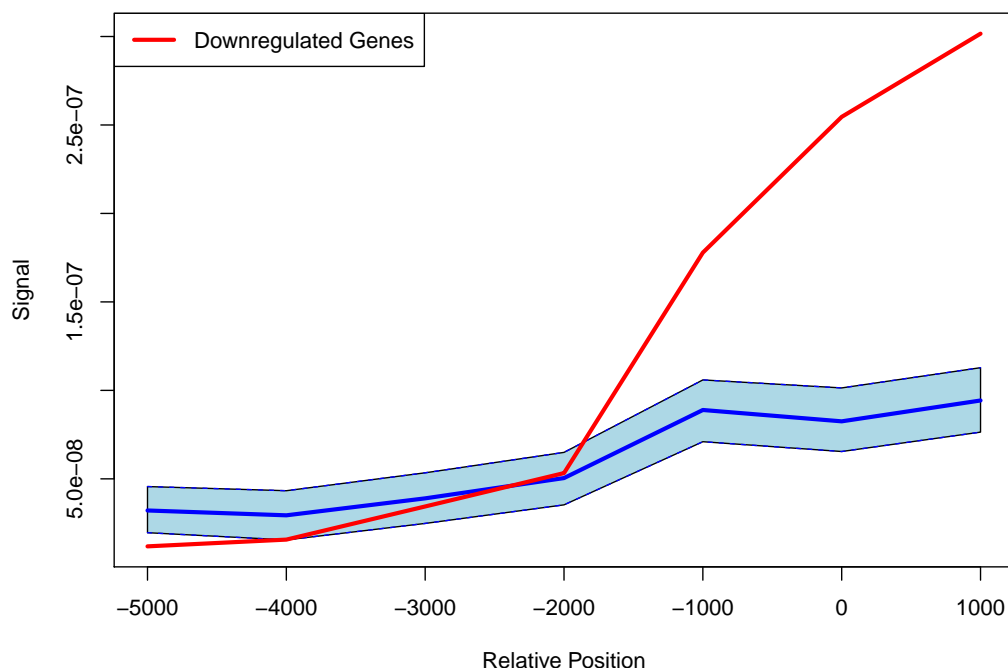
This strategy is useful for determining the location (e.g. relative to TSS) signal most often occurs relative to expression and can be coupled to ranked gene expression levels, instead of differential expression. These determined regions of interest relative to TSS can then be used in targeted analyses (e.g. `blocksStats`, see above).

## 5.2 Gene Set Enrichment

Sets of genes (e.g. genes disrupted in a certain type of cancer, or differentially expressed between experimental conditions) are ever-present in genomics research. For such genes of interest, the profile of intensities or counts can be plotted versus the profile of randomly selected gene lists using the `profilePlots` function. In the following example, the DNA methylation profile of genes silenced in cancer is significantly different to random sets of genes.

```
which.loss <- which(expr < -3)
profilePlots(prostateCvgs,
             gene.lists = list(`Downregulated Genes` = which.loss),
             cols = "red")
```

## LNCaP – PrEC



The blue region forms the “null” distribution that was created by sampling random gene lists of the same size as the user-specified gene list a number of times, as set by the `nSamples` parameter. By default, the null region is a between the 0.025 and 0.975 quantiles of the null distribution. In this example, it appears that the genes silenced in cancer have a significant gain of methylation 2000 bases either side of the TSSs, in comparison to random sets of other genes.

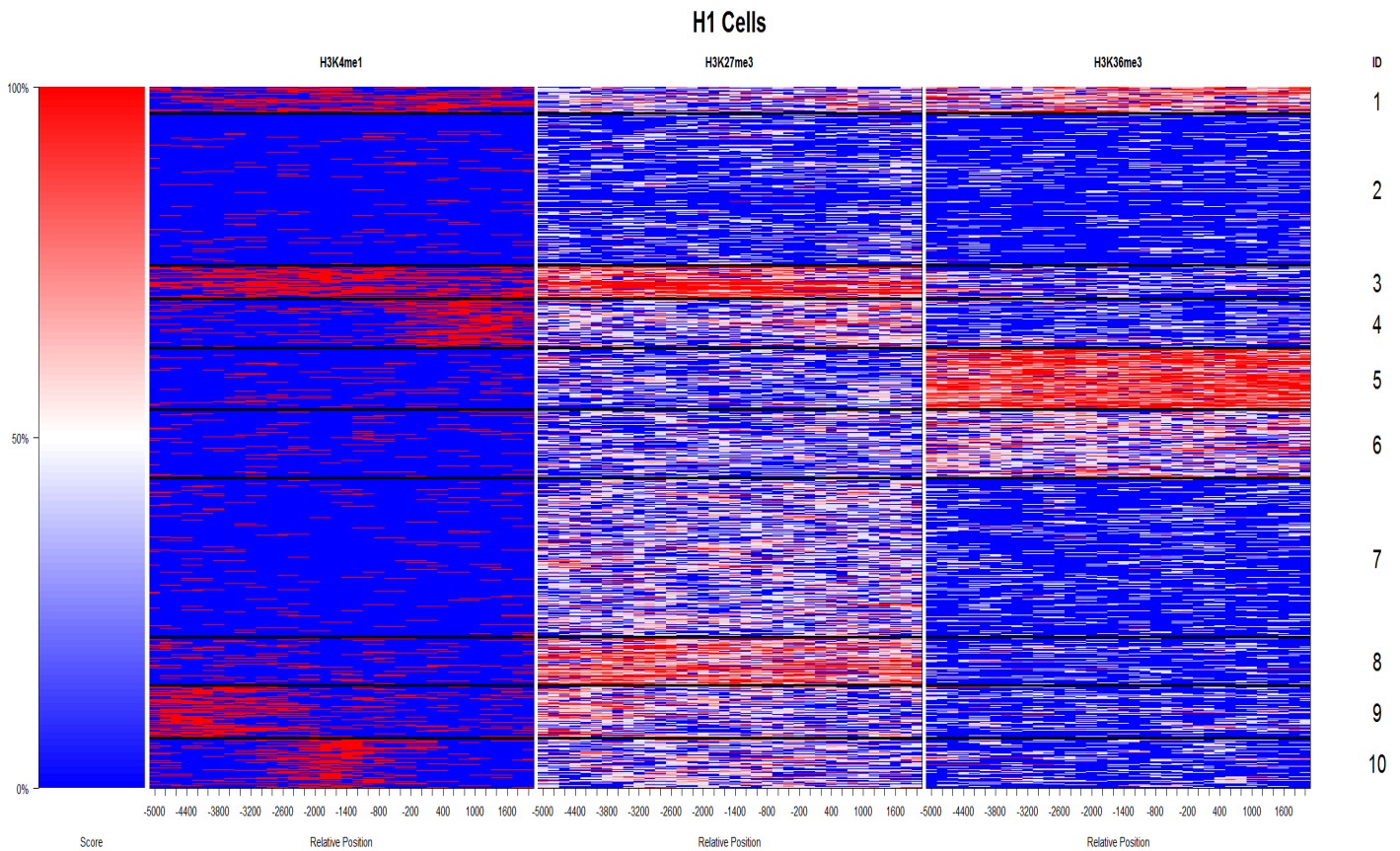
### 5.3 Clustering epigenomic signals

`clusterPlots` is another way to look at read depth at regular positions around a feature (e.g. TSS). The first step is to use `featureScores` to get the coverage tables, which essentially gives a list of coverage tables for the samples used. `clusterPlots` is then called, which does k-means clustering, or if the user wants to use their own clustering algorithm, the cluster ID of each feature can be passed in. In any case, the features are grouped by their cluster memberships and plotted as either a heatmap with one row for every feature, or a set of lineplots showing the average coverage of all features belonging to each cluster. If gene expression data is also available, it can be plotted alongside the heatmaps.

```
cvgs <- featureScores(H1samples[1:3], gene.anno, up = 5000, down = 2000, dist = "base",  
                    freq = 200, s.width = 500)
```

```
cp <- clusterPlots(cvgs, scale = function(x) sqrt(x), plot.type = "heatmap",  
                 t.name = "H1 Cells", n.clusters = 10)
```

```
null device  
1
```



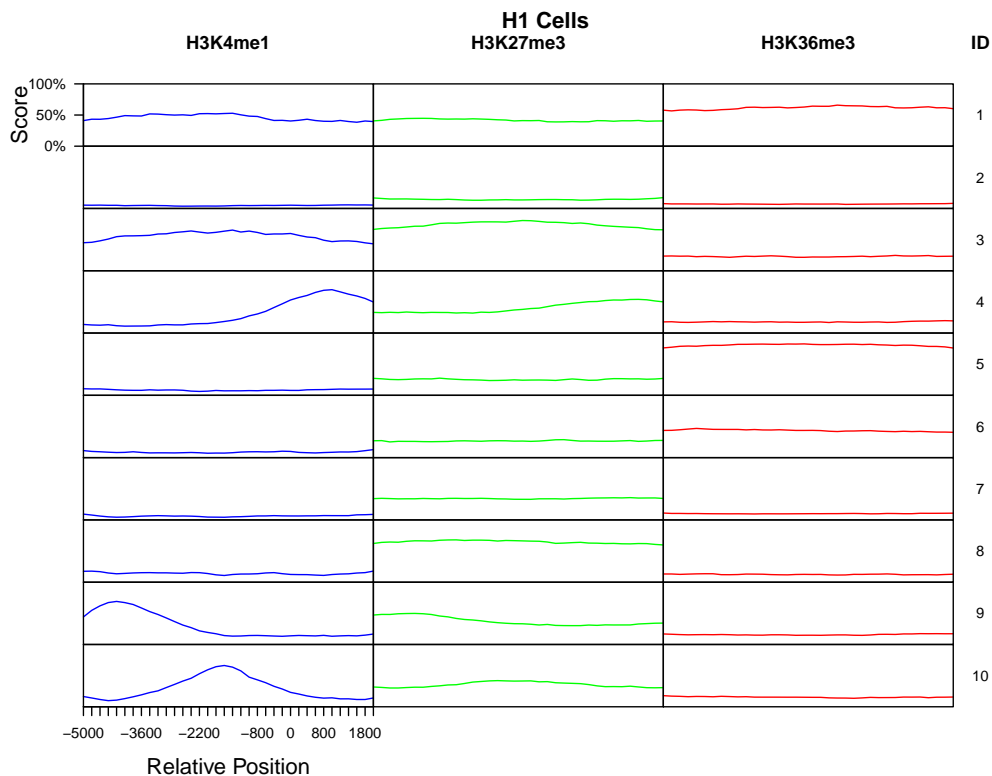
Here, we have scaled the signal using the square root transformation. If you don't specify this, no scaling is done.

Note that we have saved the output of `clusterPlots` (a `ClusteredCoverageList` object), which can be plotted in alternative ways, such as line plots:

```
table(clusters(cp))
```

```
 1    2    3    4    5    6    7    8    9   10
950 5399 1187 1770 2175 2450 5675 1698 1892 1770
```

```
clusterPlots(cp, plot.type = "line", t.name = "H1 Cells")
```



Also, this allows users to do their own clustering and use `clusterPlots` for the plotting, or to extract the cluster identifiers for downstream analyses (e.g. functional category analysis). Furthermore, in addition to specifying a vector of expression values and plotting it alongside the clustered epigenetic signal, users can give an additional vector in the `sort.data` argument to sort on within a cluster (e.g. gene length, CpG density, etc.).

## 6 Utility Functions

The function described in this section perform useful tasks that are commonly made with epigenetic data.

### 6.1 Windows and Counts

Often, it is required to create a set of windows covering the entire genome, for some analysis. The function `genomeBlocks` does this.

```

chrs <- c(50000, 10000)
names(chrs) <- c("chr1", "chr2")
genomeBlocks(chrs, width = 5000)

```

GRanges object with 12 ranges and 0 metadata columns:

```

  seqnames      ranges strand
   <Rle>        <IRanges> <Rle>
[1]   chr1 [  1, 5000]      *
[2]   chr1 [5001, 10000]   *
[3]   chr1 [10001, 15000]  *
[4]   chr1 [15001, 20000]  *

```



```

[5] chr1 [20001, 25000] *
...   ...           ...   ...
[8] chr1 [35001, 40000] *
[9] chr1 [40001, 45000] *
[10] chr1 [45001, 50000] *
[11] chr2 [ 1, 5000] *
[12] chr2 [ 5001, 10000] *

```

-----  
seqinfo: 2 sequences from an unspecified genome; no seqlengths

This example makes a `GRanges` object of 5 kb windows along both example chromosomes.

`annotationCounts` is useful to tally the counts of reads surrounding some set of genomic landmarks. `annotationBlocksCounts` is the analogous function for counting in user-specified regions of the genome.

```

annotationCounts(samples.list, head(gene.anno, 10), up = 2000, down = 500,
                 seq.len = 300)

```

	PrEC	input	PrEC	IP	LNCaP	input	LNCaP	IP
7896759		25		35		29		69
7896761		10		2		8		36
7896779		11		15		10		14
7896798		19		61		15		83
7896817		20		41		22		46
7896822		11		17		8		28
7896859		24		35		8		70
7896861		21		57		6		78
7896863		18		85		8		85
7896865		22		92		18		90

This example counts reads that fall within 2000 bases upstream and 500 bases downstream of (the first ten) TSSs in the gene annotation table. Reads are extended to 300 bases.

## 6.2 Characteristics of the DNA sequence

It would be useful to know when seeing a lack of reads in some windows, if the mappability of the window is the cause. Some regions of the genome have low complexity sequence, where reads are unlikely to map uniquely to. The function `mappabilityCalc` calculates the percentage of each region that can be mapped to by reads generated from the experiment. It operates on a user-created `BSgenome` object of a masked genome sequence. The definition of which bases are mappable and which are not depends on the read length of the sequencing technology used. Therefore, there is no one masked `BSgenome` object that can be used by all users. Note that by masking, we mean replacing the unmappable reference sequence bases by 'N', not creating a built-in mask.

```

library(BSgenome.Hsapiens36bp.UCSC.hg18mappability)
locations <- data.frame(chr = c("chr4", "chr9"),
                      position = c(50000000, 100000000))
mappabilityCalc(locations, organism = Hsapiens36bp, window = 500, type = "center")

```

```
<NA> <NA>
0.000 0.998
```

The region on chromosome 4 is completely unmappable, whereas the region on chromosome 9 is almost completely mappable.

Next, we may be interested in determining CpG density of a region.

```
cpgDensityCalc(head(gene.anno, 10), window = 100, organism = Hsapiens)
```

```
[1] 0 10 16 7 10 20 6 4 6 4
```

This example calculates the CpG density of a window 100 bases either side of the TSS for the first ten genes in the gene annotation table. By default, the CpG density is just the raw number of counts in the windows. There are also linearly, exponentially and logarithmically decaying weight schemes available.

## 7 Summary

Repitools has a number of useful functions for quality checking, analysis, and comparison of trends. Many of the functions work seamlessly on array data, as well as sequencing data. Also, there are numerous utility functions, that perform some common task in the investigation of epigenomic data. Consult the package documentation for instructions on how to use functions that were not demonstrated by this vignette.

## 8 Environment

This vignette was created in:

```
sessionInfo()
```

```
R version 2.14.1 (2011-12-22)
```

```
Platform: x86_64-pc-mingw32/x64 (64-bit)
```

```
locale:
```

```
[1] LC_COLLATE=English_Australia.1252 LC_CTYPE=English_Australia.1252
```

```
[3] LC_MONETARY=English_Australia.1252 LC_NUMERIC=C
```

```
[5] LC_TIME=English_Australia.1252
```

```
attached base packages:
```

```
[1] grid stats graphics grDevices utils datasets methods
```

```
[8] base
```

```
other attached packages:
```

```
[1] BSgenome.Hsapiens36bp.UCSC.hg18mappability_1.0
```

```
[2] cluster_1.14.1
```

```
[3] gplots_2.10.1
[4] KernSmooth_2.23-7
[5] caTools_1.12
[6] bitops_1.0-4.1
[7] gdata_2.8.2
[8] gtools_2.6.2
[9] Ringo_1.18.0
[10] Matrix_1.0-2
[11] lattice_0.20-0
[12] limma_3.10.2
[13] RColorBrewer_1.0-5
[14] Biobase_2.14.0
[15] edgeR_2.0.5
[16] BSgenome.Hsapiens.UCSC.hg18_1.3.17
[17] BSgenome_1.22.0
[18] Biostrings_2.22.0
[19] GenomicRanges_1.6.6
[20] IRanges_1.12.5
[21] Repitools_1.0.11
```

loaded via a namespace (and not attached):

```
[1] annotate_1.32.0      AnnotationDbi_1.16.0 DBI_0.2-5
[4] genefilter_1.36.0  RSQLite_0.10.0      splines_2.14.1
[7] survival_2.36-10  tools_2.14.1        xtable_1.6-0
```