

Package ‘SingleMoleculeFootprinting’

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Title Analysis tools for Single Molecule Footprinting (SMF) data

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Depends R (>= 4.4.1)

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Description SingleMoleculeFootprinting provides functions to analyze Single Molecule Footprinting (SMF) data. Following the workflow exemplified in its vignette, the user will be able to perform basic data analysis of SMF data with minimal coding effort. Starting from an aligned bam file, we show how to perform quality controls over sequencing libraries, extract methylation information at the single molecule level accounting for the two possible kind of SMF experiments (single enzyme or double enzyme), classify single molecules based on their patterns of molecular occupancy, plot SMF information at a given genomic location.

biocViews DNAMethylation, Coverage, NucleosomePositioning, DataRepresentation, Epigenetics, MethylSeq, QualityControl, Sequencing

BugReports <https://github.com/Krebslabrep/SingleMoleculeFootprinting/issues>

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Arrange_TFBSs_clusters

Convenience function to arrange a list of given TFBSs into clusters

Description

For each TFBS, the genomic neighborhood defined by max_cluster_width will be scanned for adjacent TFBSs. The hits will be filtered for min_intersite_distance where, in case of overlapping TFBSs, the second TFBS will be arbitrarily dropped. These TFBSs plus the central "anchoring" one will define a TFBS cluster. This approach implies that the same TFBS can be employed to design multiple clusters in a sliding-window fashion.

Usage

```

Arrange_TFBSs_clusters(
  TFBSs,
  max_intersite_distance = 75,
  min_intersite_distance = 15,
  max_cluster_size = 6,
  max_cluster_width = 300,
  add.single.TFs = TRUE
)
    
```

Arguments

TFBSs	GRanges object of TFBSs
max_intersite_distance	maximum allowed distance in base pairs between two TFBS centers for them to be considered part of the same cluster. Defaults to 75.
min_intersite_distance	minimum allowed distance in base pairs between two TFBS centers for them not to be discarded as overlapping. This parameter should be set according to the width of the bins used for later sorting. Defaults to 15.
max_cluster_size	maximum number of TFBSs to be contained in any given cluster. Defaults to 6
max_cluster_width	maximum width of TFBS clusters in bps. Defaults to 300
add.single.TFs	Whether to add the TFs not used to create TFBS.clusters to the list for sorting. Defaults to TRUE

Value

list with two elements: ClusterCoordinates (GRanges object of clusters coordinates) and ClusterComposition (GRangesList of sites for each cluster)

Examples

```
KLF4s = qs::qread(system.file("extdata", "KLF4_chr19.qs", package="SingleMoleculeFootprinting"))
Arrange_TFBSs_clusters(KLF4s)
```

BaitCapture	<i>Bait capture efficiency</i>
-------------	--------------------------------

Description

check bait capture efficiency. Expected to be ~70

Usage

```
BaitCapture(sampleFile, genome, baits, clObj = NULL)
```

Arguments

sampleFile	QuasR sample sheet
genome	BS genome
baits	GRanges obj of bait coordinates. We provide an example through SingleMoleculeFootprintingData::EnrichmentRegions_mm10.rds()
clObj	cluster object to employ for parallel processing created using the parallel::makeCluster function. Defaults to NULL

Value

bait capture efficiency

Examples

```
sampleFile = paste0(tempdir(), "/NRF1Pair_Qinput.txt")

if(file.exists(sampleFile)){
  library(BSgenome.Mmusculus.UCSC.mm10)
  BaitRegions = SingleMoleculeFootprintingData::EnrichmentRegions_mm10.rds()
  BaitCapture(sampleFile = sampleFile, genome = BSgenome.Mmusculus.UCSC.mm10, baits = BaitRegions)
}
```

 BinMethylation

Summarize methylation inside sorting bins

Description

Summarize methylation inside sorting bins

Usage

```
BinMethylation(MethSM, Bin)
```

Arguments

MethSM	Single molecule matrix
Bin	IRanges object with absolute coordinates for single sorting bin.

Value

Reads covering bin with their summarized methylation status

Examples

```
library(IRanges)
library(GenomicRanges)

MethSM = qs::qread(system.file("extdata", "Methylation_4.qs",
  package="SingleMoleculeFootprinting"))[[2]]$SMF_MM_TKO_DE_

TFBSs = qs::qread(system.file("extdata", "TFBSs_1.qs",
  package="SingleMoleculeFootprinting"))

motif_center_1 = start(IRanges::resize(TFBSs[1], 1, "center"))
motif_center_2 = start(IRanges::resize(TFBSs[2], 1, "center"))
SortingBins = c(
  GRanges("chr6", IRanges(motif_center_1-35, motif_center_1+25)),
```

```
GRanges("chr6", IRanges(motif_center_1-7, motif_center_1+7)),
GRanges("chr6", IRanges(motif_center_2-7, motif_center_2+7)),
GRanges("chr6", IRanges(motif_center_2+25, motif_center_2+35))
)

binMethylationValues = BinMethylation(MethSM = MethSM, Bin = SortingBins[1])
```

CallContextMethylation

Call Context Methylation

Description

Can deal with multiple samples

Usage

```
CallContextMethylation(
  sampleFile,
  samples,
  genome,
  RegionOfInterest,
  coverage = 20,
  ConvRate.thr = NULL,
  returnSM = TRUE,
  clobj = NULL,
  verbose = FALSE
)
```

Arguments

sampleFile	QuasR pointer file
samples	vector of unique sample names corresponding to the SampleName field from the sampleFile
genome	BSgenome
RegionOfInterest	GenimocRange representing the genomic region of interest
coverage	coverage threshold as integer for least number of reads to cover a cytosine for it to be carried over in the analysis. Defaults to 20.
ConvRate.thr	Convesion rate threshold. Double between 0 and 1, defaults to NULL. To skip this filtering step, set to NULL. For more information, check out the details section.
returnSM	whether to return the single molecule matrix, defaults to TRUE
clobj	cluster object for parallel processing of multiple samples. For now only used by qMeth call for bulk methylation. Should be the output of a parallel::makeCluster() call
verbose	whether to print out messages while executing. Defaults to FALSE

Details

The ConvRate.thr argument should be used with care as it could create biases (e.g. when only one C out of context is present) while generally only marginally cleaning up the data.

Value

List with two Granges objects: average methylation call (GRanges) and single molecule methylation call (matrix)

Examples

```
sampleFile = NULL
if(!is.null(sampleFile)){
Methylation <- CallContextMethylation(
  sampleFile = sampleFile,
  samples = samples,
  genome = BSgenome.Mmusculus.UCSC.mm10,
  RegionOfInterest = RegionOfInterest,
  coverage = 20,
  returnSM = TRUE,
  ConvRate.thr = NULL,
  clobj = NULL
)
}
```

cbind.fill.Matrix	<i>Implementation performing a similar operation of rbind.fill.Matrix but for columns</i>
-------------------	---

Description

Implementation performing a similar operation of rbind.fill.Matrix but for columns

Usage

```
cbind.fill.Matrix(x, y)
```

Arguments

x	sparse matrix constructed using the function Matrix::sparseMatrix. Should have Dimnames and dims (e.g. when indexing drop=FALSE)
y	sparse matrix constructed using the function Matrix::sparseMatrix. Should have Dimnames and dims (e.g. when indexing drop=FALSE)

Details

N.b. only possible fill at the moment is 0

Examples

```
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))
MethSM_1 = Methylation[[2]][[1]]
Methylation = qs::qread(system.file("extdata", "Methylation_4.qs",
package="SingleMoleculeFootprinting"))
MethSM_2 = Methylation[[2]][[1]]
cbind.fill.Matrix(MethSM_1, MethSM_2)
```

CollapseStrands	<i>Collapse strands</i>
-----------------	-------------------------

Description

Collapse strands

Usage

```
CollapseStrands(MethGR, context)
```

Arguments

MethGR	Granges obj of average methylation
context	"GC" or "HCG". Broad because indicates just the directionality of collapse.

Value

MethGR with collapsed strands (everything turned to - strand)

Examples

```
# CollapseStrands(MethGR, "GC")
```

CollapseStrandsSM *Collapse strands in single molecule matrix*

Description

The idea here is that (regardless of context) if a C is on the - strand, calling getSeq on that coord (N.b. unstranded, that's the important bit) will give a "G", a "C" if it's a + strand.

Usage

```
CollapseStrandsSM(MethSM, context, genome, chr)
```

Arguments

MethSM	Single molecule matrix
context	"GC" or "CG". Broad because indicates just the directionality of collapse.
genome	BSgenome
chr	Chromosome, MethSM doesn't carry this info

Value

Strand collapsed MethSM

Examples

```
# CollapseStrandsSM(MethSM, "GC", BSgenome.Mmusculus.UCSC.mm10, "chr19")
```

CollectCompositeData *Collect bulk SMF data for later composite plotting*

Description

Collect bulk SMF data for later composite plotting

Usage

```
CollectCompositeData(
  sampleFile,
  samples,
  genome,
  TFBSs,
  window,
  coverage = 20,
  ConvRate.thr = NULL,
  cores = 1
)
```

Arguments

sampleFile	QuasR sampleFile
samples	vector of unique sample names corresponding to the SampleName field from the sampleFile
genome	BStgenome
TFBSs	GRanges object of TF binding sites to collect info for. We recommend employing 50 to 200 TFBSs.
window	window size to collect methylation information for
coverage	coverage threshold as integer for least number of reads to cover a cytosine for it to be carried over in the analysis. Defaults to 20.
ConvRate.thr	Conversion rate threshold. Double between 0 and 1, defaults to NULL For more information, check out the details section
cores	number of cores to use

Value

data.frame of bulk SMF info ready for plotting

Examples

```

sampleFile = NULL
if(!is.null(sampleFile)){
  CollectCompositeData(
    sampleFile = sampleFile,
    samples = samples,
    genome = BStgenome.Mmusculus.UCSC.mm10,
    TFBSs = TopMotifs,
    window = 1000,
    coverage = 20,
    ConvRate.thr = NULL,
    cores = 16
  ) -> CompositeData
}

```

colMeans_drop0

Calculate colMeans after dropping zeros

Description

Calculate colMeans after dropping zeros

Usage

```
colMeans_drop0(MethSM)
```

Arguments

MethSM one single molecule sparse matrix

Value

colMeans (N.b. this is +1 based)

CompositeMethylationCorrelation
Composite Methylation Rate

Description

Monitor methylation rate distribution in a low coverage samples as compared to a high coverage "reference" one. It bins cytosines with similar methylation rates (as observed in the HighCoverage sample) into bins. A single methylation rate value is computed for each bin

Usage

```
CompositeMethylationCorrelation(
  LowCoverage,
  LowCoverage_samples,
  HighCoverage,
  HighCoverage_samples,
  bins = 50,
  returnDF = FALSE,
  returnPlot = TRUE,
  RMSE = TRUE,
  return_RMSE_DF = FALSE,
  return_RMSE_plot = TRUE
)
```

Arguments

LowCoverage Single GRanges object as returned by CallContextMethylation function run with Coverage parameter set to 1. The object can also contain cytosines from multiple contexts

LowCoverage_samples Samples to use from the LowCoverage object. Either a string or a vector (for multiple samples).

HighCoverage Single GRanges object as returned by CallContextMethylation function. The object can also contain cytosines from multiple contexts.

HighCoverage_samples Single sample to use from HighCoverage. String

bins The number of bins for which to calculate the "binned" methylation rate. Defaults to 50

returnDF	Whether to return the data.frame used for plotting. Defaults to FALSE
returnPlot	Whether to return the plot. Defaults to TRUE
RMSE	Whether to calculate Mean squared error (RMSE) of methylation rate distribution estimates for low coverage samples. Defaults to TRUE
return_RMSE_DF	Whether to return a data.frame of computed RMSE values. Defaults to FALSE
return_RMSE_plot	Whether to return a barplot of computed values. Defaults to TRUE

Examples

```
# I don't have enough example data for this
# CompositeMethylationCorrelation(LowCoverage = LowCoverage$DGCHN,
#                                 LowCoverage_samples = LowCoverage_Samples,
#                                 HighCoverage = HighCoverage$DGCHN,
#                                 HighCoverage_samples = HighCoverage_samples[1],
#                                 returnDF = FALSE,
#                                 returnPlot = TRUE,
#                                 RMSE = TRUE,
#                                 return_RMSE_DF = FALSE,
#                                 return_RMSE_plot = TRUE)
```

CompositePlot *Plot composite SMF data*

Description

Will use geom_point with ≤ 5000 points, geom_hex otherwise

Usage

```
CompositePlot(CompositeData, span = 0.1, TF)
```

Arguments

CompositeData	the output of the CollectCompositeData function
span	the span parameter to pass to geom_smooth
TF	string of TF name to use for plot title

Examples

```
# CompositePlot(CompositeData = CompositeData, span = 0.1, TF = "Rest")
```

ConversionRate	<i>Conversion rate</i>
----------------	------------------------

Description

calculate sequencing library conversion rate on a chromosome of choice

Usage

```
ConversionRate(sampleFile, genome, chr = 19, cores = 1)
```

Arguments

sampleFile	QuasR sample sheet
genome	BS genome
chr	chromosome to calculate conversion rate on (default: 19)
cores	number of cores for parallel processing. Defaults to 1

Examples

```
# ConversionRate(sampleFile = sampleFile,  
# genome = BSgenome.Mmusculus.UCSC.mm10, chr = 19, cores = 1)
```

CoverageFilter	<i>Filter Cs for coverage</i>
----------------	-------------------------------

Description

Filter Cs for coverage

Usage

```
CoverageFilter(MethGR, thr)
```

Arguments

MethGR	Granges obj of average methylation
thr	converage threshold

Value

filtered MethGR

Create_MethylationCallingWindows

Create methylation calling windows to call context methylation in one run for clusters lying proximally to each other

Description

Relevant for genome-wide analyses

Usage

```
Create_MethylationCallingWindows(
  RegionsOfInterest,
  max_intercluster_distance = 1e+05,
  max_window_width = 5e+06,
  min_cluster_width = 600,
  genomic.seqlenghts,
  fix.window.size = FALSE,
  max.window.size = 500
)
```

Arguments

RegionsOfInterest
TFBS cluster coordinates analogous to ClusterCoordinates object returned by Arrange_TFBSs_clusters function

max_intercluster_distance
maximum distance between two consecutive TFBS clusters for them to be grouped in the same window

max_window_width
upper limit to window width. This value should be adjusted according to the user's system as it determines the amount of memory used in the later context methylation call

min_cluster_width
lower limit to window width. Corresponds to the scenario when a window contains a single TFBS cluster.

genomic.seqlenghts
used to fix the windows spanning over chromosome edges. To be fetched by GenomeInfoDb::seqlengths() or equivalent.

fix.window.size
Defaults to FALSE. When TRUE, overrides arguments max_intercluster_distance and max_window_width and produces windows containing a fixed number of TFBS_clusters.

max.window.size
Max number of TFBS_clusters per window. Used only when fix.window.size is TRUE. N.b.: window size could be slightly higher than passed value if RegionsOfInterest overlap

Value

GRanges object of window coordinates to be used for more efficient calls of CallContextMethylation

Examples

```
KLF4s = qs::qread(system.file("extdata", "KLF4_chr19.qs", package="SingleMoleculeFootprinting"))
Create_MethylationCallingWindows(RegionsOfInterest = KLF4s)
```

DetectExperimentType *Detect type of experiment*

Description

Detect type of experiment

Usage

```
DetectExperimentType(Samples)
```

Arguments

Samples SampleNames field from QuasR sampleFile

Examples

```
CacheDir = ExperimentHub::getExperimentHubOption(arg = "CACHE")
sampleFile = paste0(CacheDir, "/NRF1Pair_sampleFile.txt")
samples = suppressMessages(unique(readr::read_delim(sampleFile, delim = "\t")[[2]]))
DetectExperimentType(samples)
```

FilterByConversionRate
Calculate reads conversion rate

Description

Calculate reads conversion rate

Usage

```
FilterByConversionRate(MethSM, chr, genome, thr)
```

Arguments

MethSM	as comes out of the func GetSingleMolMethMat
chr	Chromosome, MethSM doesn't carry this info
genome	BSgenome
thr	Double between 0 and 1. Threshold below which to filter reads.

Value

Filtered MethSM

Examples

```
library(BSgenome.Mmusculus.UCSC.mm10)
MethSM = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))[[2]]$SMF_MM_TKO_DE_
FilterByConversionRate(MethSM, chr = "chr19",
genome = BSgenome.Mmusculus.UCSC.mm10, thr = 0.8)
```

FilterContextCytosines

Filter Cytosines in context

Description

Filter Cytosines in context

Usage

```
FilterContextCytosines(MethGR, genome, context)
```

Arguments

MethGR	Granges obj of average methylation
genome	BSgenome
context	Context of interest (e.g. "GC", "CG",...)

Value

filtered Granges obj

Examples

```
library(BSgenome.Mmusculus.UCSC.mm10)
MethGR = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))[[1]]

FilterContextCytosines(MethGR, BSgenome.Mmusculus.UCSC.mm10, "NGCNN")
```

 filter_reads_from_MethGR

*Recalculate *_T and *_M values in MethGR object after filtering reads e.g. for conversion rate*

Description

Recalculate *_T and *_M values in MethGR object after filtering reads e.g. for conversion rate

Usage

```
filter_reads_from_MethGR(MethGR, MethSM, MethSM_filtered, sampleIndex)
```

Arguments

MethGR	GRanges object of methylation call
MethSM	Single Molecule methylation matrix
MethSM_filtered	Single Molecule methylation matrix after filtering reads
sampleIndex	index for sample to treat. It serves as a correspondence between the index of the SM matrix and the order samples appear in the elementMetadata() columns

Value

MethGR with recalculated counts

full.join.granges	<i>Utility function to perform the dplyr full_join operation on GRanges object</i>
-------------------	--

Description

Utility function to perform the dplyr full_join operation on GRanges object

Usage

```
full.join.granges(MethGR1, MethGR2)
```

Arguments

MethGR1	Methylation GRanges as output by the CallContextMethylation() function
MethGR2	Methylation GRanges as output by the CallContextMethylation() function

GetQuasRprj	<i>Get QuasRprj</i>
-------------	---------------------

Description

Get QuasRprj

Usage

```
GetQuasRprj(sampleFile, genome)
```

Arguments

sampleFile	QuasR pointer file
genome	BSgenome

Examples

```
library(BSgenome.Mmusculus.UCSC.mm10)
CacheDir <- ExperimentHub::getExperimentHubOption(arg = "CACHE")
sampleFile = paste0(CacheDir, "/NRF1Pair_sampleFile.txt")
QuasRprj = GetQuasRprj(sampleFile, BSgenome.Mmusculus.UCSC.mm10)
```

GetSingleMolMethMat	<i>Get Single Molecule methylation matrix</i>
---------------------	---

Description

Used internally as the first step in CallContextMethylation

Usage

```
GetSingleMolMethMat(QuasRprj, range, sample)
```

Arguments

QuasRprj	QuasR project object as returned by calling the QuasR function qAlign on previously aligned data
range	GenimocRange representing the genomic region of interest
sample	One of the sample names as reported in the SampleName field of the QuasR sampleFile provided to qAlign. N.b. all the files with the passed sample name will be used to call methylation

Value

List of single molecule methylation matrixes (all Cytosines), one per sample

Examples

```
library(BSgenome.Mmusculus.UCSC.mm10)
library(IRanges)
library(GenomicRanges)

CacheDir <- ExperimentHub::getExperimentHubOption(arg = "CACHE")
sampleFile = paste0(CacheDir, "/NRF1Pair_sampleFile.txt")
sample = suppressMessages(readr::read_delim(sampleFile, delim = "\t")[[2]])
QuasRprj = GetQuasRprj(sampleFile, BSgenome.Mmusculus.UCSC.mm10)
range = GRanges("chr6", IRanges(88106000, 88106500))

GetSingleMolMethMat(QuasRprj, range, sample)
```

GRanges_to_DF

Manipulate GRanges into data.frame

Description

Inner utility for LowCoverageMethRateDistribution

Usage

```
GRanges_to_DF(GRanges_obj)
```

Arguments

GRanges_obj GRanges object as returned by CallContextMethylation function

HierarchicalClustering

Perform Hierarchical clustering on single reads

Description

Perform Hierarchical clustering on single reads

Usage

```
HierarchicalClustering(MethSM)
```

Arguments

MethSM Single molecule methylation matrix

LowCoverageMethRate_RMSE

Low Coverage Methylation Rate RMSE

Description

Calculate Root mean squared error (RMSE) of methylation rate distribution estimates for low coverage samples

Usage

```
LowCoverageMethRate_RMSE(BinnedMethRate)
```

Arguments

BinnedMethRate data.frame as returned by GRanges_to_DF function.

MaskSNPs

Utility function to remove cytosines whose MTase target genomic context is affected by SNPs

Description

Utility function to remove cytosines whose MTase target genomic context is affected by SNPs

Usage

```
MaskSNPs(
  Methylation,
  CytosinesToMask,
  MaskSMmat = FALSE,
  SampleStringMatch = list(Cast = "_CTKO", Spret = "_STKO"),
  Experiment
)
```

Arguments

Methylation as output by the CallContextMethylation() function

CytosinesToMask

GRanges specifying the coordinate of the cytosines to discard.

MaskSMmat

whether the parameter Methylation includes single molecule matrixes

SampleStringMatch

list of per-sample string matches that are used to uniquely identify the relevant column for each species in the Methylation object. Defaults to list(Cast = "_CTKO", Spret = "_STKO")

Experiment

as detected by the DetectExperimentType() function. Should be either "DE" or "NO"

Examples

```

Methylation = qs::qread(system.file("extdata", "Methylation_2.qs",
package="SingleMoleculeFootprinting"))
CytosinesToMask = qs::qread(system.file("extdata", "cytosines_to_mask.qs",
package="SingleMoleculeFootprinting"))

MaskSNPs(Methylation = Methylation, CytosinesToMask = CytosinesToMask, MaskSmmat = FALSE,
SampleStringMatch = list(Cast = "_CTK0", Spret = "_STK0"), Experiment = "DE") -> Methylation_masked

```

MethSM.to.MethGR	<i>Compute MethGR from MethSM</i>
------------------	-----------------------------------

Description

Compute MethGR from MethSM

Usage

```
MethSM.to.MethGR(MethSM, chromosome)
```

Arguments

MethSM	internal CallContextMethylation
chromosome	string

panel.cor	<i>Utility for HighCoverage_MethRate_SampleCorrelation</i>
-----------	--

Description

Utility for HighCoverage_MethRate_SampleCorrelation

Usage

```
panel.cor(x, y, digits = 2, prefix = "", cex.cor)
```

Arguments

x	x variable
y	y variable
digits	number of digits
prefix	string
cex.cor	graphical param

`panel.hist`*Utility for HighCoverage_MethRate_SampleCorrelation*

Description

Utility for HighCoverage_MethRate_SampleCorrelation

Usage`panel.hist(x, ...)`**Arguments**

<code>x</code>	data for hist
<code>...</code>	data for hist

`panel.jet`*Utility for HighCoverage_MethRate_SampleCorrelation*

Description

Utility for HighCoverage_MethRate_SampleCorrelation

Usage`panel.jet(...)`**Arguments**

<code>...</code>	data for lower pairs panel
------------------	----------------------------

PlotAvgSMF	<i>Plot average methylation</i>
------------	---------------------------------

Description

Plot average methylation

Usage

```
PlotAvgSMF(
  MethGR,
  MethSM = NULL,
  RegionOfInterest,
  SortedReads = NULL,
  ShowContext = FALSE,
  TFBSs = NULL,
  SNPs = NULL,
  SortingBins = NULL
)
```

Arguments

MethGR	Average methylation GRanges obj
MethSM	Single molecule matrix(es)
RegionOfInterest	GRanges interval to plot
SortedReads	List of sorted reads, needs to be passed along with the parameter MethSM. If both are passed, only counts relevant to sorting will be plotted
ShowContext	TRUE or FALSE (default). Causes the genomic context of the plotted cytosines to be displayed as the dot shape
TFBSs	GRanges object of transcription factor binding sites to include in the plot. Assumed to be already subset. Also assumed that the tf names are under the column "TF"
SNPs	GRanges object of SNPs to visualize. Assumed to be already subset. Assumed to have the reference and alternative sequences respectively under the columns "R" and "A"
SortingBins	GRanges object of sorting bins (absolute) coordinate to visualize

Examples

```
library(GenomicRanges)

RegionOfInterest = GRanges("chr12", IRanges(20464551, 20465050))
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))
```

```
TFBSs = qs::qread(system.file("extdata", "TFBSs_3.qs", package="SingleMoleculeFootprinting"))
PlotAvgSMF(MethGR = Methylation[[1]], RegionOfInterest = RegionOfInterest, TFBSs = TFBSs)
```

PlotSingleMoleculeStack
Plot single molecule stack

Description

Plot single molecule stack

Usage

```
PlotSingleMoleculeStack(MethSM, RegionOfInterest)
```

Arguments

MethSM Single molecule methylation matrix
RegionOfInterest
 GRanges interval to plot

Examples

```
library(GenomicRanges)

RegionOfInterest = GRanges("chr12", IRanges(20464551, 20465050))
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))

PlotSingleMoleculeStack(MethSM = Methylation[[2]], RegionOfInterest = RegionOfInterest)
```

PlotSingleSiteSMF *Plot SMF data at single site*

Description

Plot SMF data at single site

Usage

```
PlotSingleSiteSMF(
  Methylation,
  RegionOfInterest,
  ShowContext = FALSE,
  TFBSs = NULL,
  SNPs = NULL,
  SortingBins = NULL,
  SortedReads = NULL,
  sorting.strategy = "None"
)
```

Arguments

Methylation	Context methylation object as returned by CallContextMethylation function
RegionOfInterest	GRanges interval to plot
ShowContext	TRUE or FALSE (default). Causes the genomic context of the plotted cytosines to be displayed as the dot shape
TFBSs	GRanges object of transcription factor binding sites to include in the plot. Assumed to be already subset. Also assumed that the tf names are under the column "TF"
SNPs	GRanges object of SNPs to visualize. Assumed to be already subset. Assumed to have the reference and alternative sequences respectively under the columns "R" and "A"
SortingBins	GRanges object of sorting bins (absolute) coordinate to visualize
SortedReads	Defaults to NULL, in which case will plot unsorted reads. Sorted reads object as returned by SortReads function or "HC" to perform hierarchical clustering
sorting.strategy	One of "classical" (default), "custom", "hierarchical.clustering" or "None". Determines how to display reads. For details check documentation from PlotSM function.

Examples

```
library(GenomicRanges)

RegionOfInterest = GRanges("chr12", IRanges(20464551, 20465050))
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))
TFBSs = qs::qread(system.file("extdata", "TFBSs_3.qs", package="SingleMoleculeFootprinting"))
SortedReads = SortReadsBySingleTF(MethSM = Methylation[[2]], TFBS = TFBSs)

PlotSingleSiteSMF(Methylation = Methylation,
  RegionOfInterest = RegionOfInterest,
  SortedReads = SortedReads,
  TFBSs = TFBSs)
```

PlotSM

*Wrapper for PlotSingleMoleculeStack function***Description**

adds the convenience of arranging reads before plotting

Usage

```
PlotSM(
  MethSM,
  RegionOfInterest,
  sorting.strategy = "classical",
  SortedReads = NULL
)
```

Arguments

MethSM	Single molecule methylation matrix
RegionOfInterest	GRanges interval to plot
sorting.strategy	One of "classical" (default), "custom", "hierarchical.clustering" or "None". Set to "classical" for classical one-TF/TF-pair sorting (as described in Sönmezer et al, MolCell, 2021). Should be passed along with argument SortedReads set to the Sorted reads object as returned by SortReads function. If set to "custom", SortedReads should be a list with one item per sample (corresponding to MethSM). If set to "hierarchical.clustering", the function will perform hierarchical clustering in place on a subset of reads. Useful to check for duplicated reads in amplicon sequencing experiments. If set to "None", it will plot unsorted reads. The argument sorting.strategy will always determine how to display reads with priority over the argument SortedReads
SortedReads	Defaults to NULL, in which case will plot unsorted reads. Sorted reads object as returned by SortReads function

Examples

```
library(GenomicRanges)

RegionOfInterest = GRanges("chr12", IRanges(20464551, 20465050))
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))
TFBSs = qs::qread(system.file("extdata", "TFBSs_3.qs",
package="SingleMoleculeFootprinting"))
SortedReads = SortReadsBySingleTF(MethSM = Methylation[[2]], TFBS = TFBSs)

PlotSM(MethSM = Methylation[[2]], RegionOfInterest = RegionOfInterest, SortedReads = SortedReads)
```

Plot_LowCoverageMethRate

Plot low coverage methylation rate

Description

Inner utility for LowCoverageMethRateDistribution

Usage

Plot_LowCoverageMethRate(Plotting_DF)

Arguments

Plotting_DF data.frame as returned by GRanges_to_DF function.

Plot_LowCoverageMethRate_RMSE

Plot Low Coverage Methylation Rate RMSE

Description

Produce barplot of RMSE values calculated for methylation rate distribution estimates of low coverage samples

Usage

Plot_LowCoverageMethRate_RMSE(RMSE_DF)

Arguments

RMSE_DF data.frame as returned by the LowCoverageMethRate_RMSE function

<code>rbind.fill.Matrix</code>	<i>Implementation performing a similar operation of <code>plyr::rbind.fill.matrix</code> but for <code>sparseMatrix</code></i>
--------------------------------	--

Description

Implementation performing a similar operation of `plyr::rbind.fill.matrix` but for `sparseMatrix`

Usage

```
rbind.fill.Matrix(x, y)
```

Arguments

<code>x</code>	sparse matrix constructed using the function <code>Matrix::sparseMatrix</code> . Should have <code>Dimnames</code> and <code>dims</code> (e.g. when indexing <code>drop=FALSE</code>)
<code>y</code>	sparse matrix constructed using the function <code>Matrix::sparseMatrix</code> . Should have <code>Dimnames</code> and <code>dims</code> (e.g. when indexing <code>drop=FALSE</code>)

Details

N.b. only possible fill at the moment is 0

Examples

```
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))
MethSM_1 = Methylation[[2]][[1]]
Methylation = qs::qread(system.file("extdata", "Methylation_4.qs",
package="SingleMoleculeFootprinting"))
MethSM_2 = Methylation[[2]][[1]]
rbind.fill.Matrix(MethSM_1, MethSM_2)
```

<code>rowMeans_drop0</code>	<i>Calculate rowMeans after dropping zeros</i>
-----------------------------	--

Description

Calculate rowMeans after dropping zeros

Usage

```
rowMeans_drop0(MethSM)
```

Arguments

MethSM one single molecule sparse matrix

Value

rowMeans (N.b. this is +1 based)

SingleTFStateQuantificationPlot
Single TF state quantification bar

Description

Single TF state quantification bar

Usage

SingleTFStateQuantificationPlot(SortedReads)

Arguments

SortedReads Sorted reads as returned by SortReadsBySingleTF

SingleTFStates *Hard-coded interpretation of biological states from single TF sorting*

Description

Hard-coded interpretation of biological states from single TF sorting

Usage

SingleTFStates()

Value

list of states

Examples

SingleTFStates()

SortReads	<i>Sort reads by single TF</i>
-----------	--------------------------------

Description

Sort reads by single TF

Usage

```
SortReads(MethSM, BinsCoordinates, coverage = NULL)
```

Arguments

MethSM	Single molecule matrix
BinsCoordinates	IRanges object of absolute coordinates for sorting bins
coverage	integer. Minimum number of reads covering all sorting bins for sorting to be performed

Value

list of sorted reads

Examples

```
library(IRanges)

Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))
TFBS = qs::qread(system.file("extdata", "TFBSs_3.qs",
package="SingleMoleculeFootprinting"))

bins = list(c(-35,-25), c(-15,15), c(25,35))
TFBS_center = start(TFBS) + (end(TFBS)-start(TFBS))/2
BinsCoordinates = IRanges(
start = c(TFBS_center+bins[[1]][1], TFBS_center+bins[[2]][1], TFBS_center+bins[[3]][1]),
end = c(TFBS_center+bins[[1]][2], TFBS_center+bins[[2]][2], TFBS_center+bins[[3]][2])
)

SortedReads = SortReads(Methylation[[2]]$SMF_MM_TKO_DE_, BinsCoordinates, coverage = 20)
```

SortReadsBySingleTF *Wrapper to SortReads for single TF case*

Description

Wrapper to SortReads for single TF case

Usage

```
SortReadsBySingleTF(  
  MethSM,  
  TFBS,  
  bins = list(c(-35, -25), c(-15, 15), c(25, 35)),  
  coverage = 20  
)
```

Arguments

MethSM	Single molecule matrix list as returned by CallContextMethylation
TFBS	Transcription factor binding site to use for sorting, passed as a GRanges object of length 1
bins	list of 3 relative bin coordinates. Defaults to list(c(-35,-25), c(-15,15), c(25,35)). bins[[1]] represents the upstream bin, with coordinates relative to the start of the TFBS. bins[[2]] represents the TFBS bin, with coordinates relative to the center of the TFBS. bins[[3]] represents the downstream bin, with coordinates relative to the end of the TFBS.
coverage	integer. Minimum number of reads covering all sorting bins for sorting to be performed. Defaults to 20

Value

List of reads sorted by single TF

Examples

```
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",  
  package="SingleMoleculeFootprinting"))  
TFBSs = qs::qread(system.file("extdata", "TFBSs_3.qs",  
  package="SingleMoleculeFootprinting"))  
  
SortedReads = SortReadsBySingleTF(MethSM = Methylation[[2]], TFBS = TFBSs)
```

SortReadsBySingleTF_MultiSiteWrapper

Convenience wrapper to sort single molecule according to TFBS clusters at multiple sites in the genome

Description

The function starts from a list of single TFBSs, arranges them into clusters, calls methylation at the interested sites and outputs sorted reads

Usage

```
SortReadsBySingleTF_MultiSiteWrapper(
  sampleFile,
  samples,
  genome,
  coverage = 20,
  ConvRate.thr = NULL,
  CytosinesToMask = NULL,
  TFBSs,
  max_interTF_distance = 1e+05,
  max_window_width = 5e+06,
  min_cluster_width = 600,
  fix.window.size = FALSE,
  max.window.size = NULL,
  sorting_coverage = 30,
  bins = list(c(-35, -25), c(-15, 15), c(25, 35)),
  cores = 1
)
```

Arguments

sampleFile	QuasR pointer file
samples	samples to use, from the SampleName field of the sampleFile
genome	BSgenome
coverage	coverage threshold as integer for least number of reads to cover a cytosine for it to be carried over in the analysis. Defaults to 20.
ConvRate.thr	Conversion rate threshold. Double between 0 and 1, defaults to NULL. To skip this filtering step, set to NULL. For more information, check out the details section.
CytosinesToMask	CytosinesToMask object. Passed to MaskSNPs function
TFBSs	GRanges object of transcription factor binding sites coordinates
max_interTF_distance	maximum distance between two consecutive TFBSs for them to be grouped in the same window

<code>max_window_width</code>	upper limit to window width. This value should be adjusted according to the user's system as it determines the amount of memory used in the later context methylation call
<code>min_cluster_width</code>	lower limit to window width. Corresponds to the scenario when a window contains a single TFBS.
<code>fix.window.size</code>	defaults to FALSE. Passed to <code>Create_MethylationCallingWindows</code> function.
<code>max.window.size</code>	defaults to NULL. Passed to <code>Create_MethylationCallingWindows</code> function.
<code>sorting_coverage</code>	integer. Minimum number of reads covering all sorting bins for sorting to be performed. Defaults to 30.
<code>bins</code>	list of 3 relative bin coordinates. Defaults to <code>list(c(-35,-25), c(-15,15), c(25,35))</code> . <code>bins[[1]]</code> represents the upstream bin, with coordinates relative to the start of the most upstream TFBS. <code>bins[[2]]</code> represents all the TFBS bins, with coordinates relative to the center of each TFBS. <code>bins[[3]]</code> represents the downstream bin, with coordinates relative to the end of the most downstream TFBS.
<code>cores</code>	number of cores to use for parallel processing of multiple Methylation Calling Windows (i.e. groupings of adjacent TFBS clusters)

Value

list where `[[1]]` is the TFBSs GRanges object describing coordinates TFBSs used to sort single molecules `[[2]]` is a list of SortedReads nested per TFBS_cluster and sample `[[3]]` is a tibble reporting the count (and frequency) of reads per state, sample and TFBS cluster

Examples

```
sampleFile = NULL
if(!is.null(sampleFile)){
  SortReadsBySingleTF_MultiSiteWrapper(
    sampleFile = sampleFile,
    samples = samples,
    genome = BSgenome.Mmusculus.UCSC.mm10,
    coverage = 20, ConvRate.thr = NULL,
    CytosinesToMask = NULL,
    TFBSs = KLF4s,
    max_interTF_distance = NULL, max_window_width = NULL, min_cluster_width = NULL,
    fix.window.size = TRUE, max.window.size = 50,
    cores = 4
  ) -> sorting_results
}
```

SortReadsByTFCluster *Wrapper to SortReads for TF cluster case*

Description

Wrapper to SortReads for TF cluster case

Usage

```
SortReadsByTFCluster(
  MethSM,
  TFBS_cluster,
  bins = list(c(-35, -25), c(-7, 7), c(25, 35)),
  coverage = 30
)
```

Arguments

MethSM	Single molecule matrix list as returned by CallContextMethylation
TFBS_cluster	Transcription factor binding sites to use for sorting, passed as a GRanges object of length > 1
bins	list of 3 relative bin coordinates. Defaults to list(c(-35,-25), c(-7,7), c(25,35)). bins[[1]] represents the upstream bin, with coordinates relative to the start of the most upstream TFBS. bins[[2]] represents all the TFBS bins, with coordinates relative to the center of each TFBS. bins[[3]] represents the downstream bin, with coordinates relative to the end of the most downstream TFBS.
coverage	integer. Minimum number of reads covering all sorting bins for sorting to be performed. Defaults to 30

Value

List of reads sorted by TF cluster

Examples

```
Methylation = qs::qread(system.file("extdata", "Methylation_4.qs",
  package="SingleMoleculeFootprinting"))
TFBSs = qs::qread(system.file("extdata", "TFBSs_1.qs",
  package="SingleMoleculeFootprinting"))
```

```
SortedReads = SortReadsByTFCluster(MethSM = Methylation[[2]], TFBS_cluster = TFBSs)
```

 SortReadsByTFCluster_MultiSiteWrapper

Convenience wrapper to sort single molecule according to TFBS clusters at multiple sites in the genome

Description

The function starts from a list of single TFBSs, arranges them into clusters, calls methylation at the interested sites and outputs sorted reads

Usage

```
SortReadsByTFCluster_MultiSiteWrapper(
  sampleFile,
  samples,
  genome,
  coverage = 20,
  ConvRate.thr = 0.8,
  CytosinesToMask = NULL,
  TFBSs,
  max_intersite_distance = 75,
  min_intersite_distance = 15,
  max_cluster_size = 10,
  max_cluster_width = 300,
  add.single.TFs = TRUE,
  max_intercluster_distance = 1e+05,
  max_window_width = 5e+06,
  min_cluster_width = 600,
  fix.window.size = FALSE,
  max.window.size = NULL,
  sorting_coverage = 30,
  bins = list(c(-35, -25), c(-7, 7), c(25, 35)),
  cores = 1
)
```

Arguments

sampleFile	QuasR pointer file
samples	samples to use, from the SampleName field of the sampleFile
genome	BSgenome
coverage	coverage threshold as integer for least number of reads to cover a cytosine for it to be carried over in the analysis. Defaults to 20.
ConvRate.thr	Conversion rate threshold. Double between 0 and 1, defaults to 0.8. To skip this filtering step, set to NULL. For more information, check out the details section.
CytosinesToMask	CytosinesToMask object. Passed to MaskSNPs function

TFBSs	GRanges object of transcription factor binding sites coordinates
max_intersite_distance	maximum allowed distance in base pairs between two TFBS centers for them to be considered part of the same cluster. Defaults to 75.
min_intersite_distance	minimum allowed distance in base pairs between two TFBS centers for them not to be discarded as overlapping. This parameter should be set according to the width of the bins used for later sorting. Defaults to 15.
max_cluster_size	maximum number of TFBSs to be contained in any given cluster. Defaults to 10
max_cluster_width	maximum cluster width in bp. Defaults to 300
add.single.TFs	whether to add to output the TFBSs that didn't make it into clusters. Defaults to TRUE
max_intercluster_distance	maximum distance between two consecutive TFBS clusters for them to be grouped in the same window
max_window_width	upper limit to window width. This value should be adjusted according to the user's system as it determines the amount of memory used in the later context methylation call
min_cluster_width	lower limit to window width. Corresponds to the scenario when a window contains a single TFBS cluster.
fix.window.size	defaults to FALSE. Passed to Create_MethylationCallingWindows function.
max.window.size	defaults to NULL. Passed to Create_MethylationCallingWindows function.
sorting_coverage	integer. Minimum number of reads covering all sorting bins for sorting to be performed. Defaults to 30.
bins	list of 3 relative bin coordinates. Defaults to list(c(-35,-25), c(-7,7), c(25,35)). bins[[1]] represents the upstream bin, with coordinates relative to the start of the most upstream TFBS. bins[[2]] represents all the TFBS bins, with coordinates relative to the center of each TFBS. bins[[3]] represents the downstream bin, with coordinates relative to the end of the most downstream TFBS.
cores	number of cores to use for parallel processing of multiple Methylation Calling Windows (i.e. groupings of adjacent TFBS clusters)

Value

list where [[1]] is the TFBS_Clusters object describing coordinates and composition of the TFBS clusters used to sort single molecules [[2]] is a list of SortedReads nested per TFBS_cluster and sample [[3]] is a tibble reporting the count (and frequency) of reads per state, samples and TFBS cluster

Examples

```

sampleFile = NULL
if(!is.null(sampleFile)){
SortReadsByTFCluster_MultiSiteWrapper(
sampleFile = sampleFile,
samples = samples,
genome = BSgenome.Mmusculus.UCSC.mm10,
coverage = 20, ConvRate.thr = NULL,
CytosinesToMask = NULL,
TFBSs = KLF4s,
max_interTF_distance = NULL, max_window_width = NULL, min_cluster_width = NULL,
fix.window.size = TRUE, max.window.size = 50,
cores = 4
) -> sorting_results
}

```

StateQuantification *Convenience for calculating state frequencies*

Description

Convenience for calculating state frequencies

Usage

```
StateQuantification(SortedReads, states)
```

Arguments

SortedReads	List of sorted reads (can be multiple samples) as returned by either read sorting function (SortReads, SortReadsBySingleTF, SortReadsByTFCluster)
states	states reporting the biological interpretation of patterns as return by either SingleTFStates or TFPairStates functions. If NULL (default) will return frequencies without biological interpretation.

Value

tibble with state frequency information

Examples

```

Methylation = qs::qread(system.file("extdata", "Methylation_4.qs",
package="SingleMoleculeFootprinting"))
TFBSs = qs::qread(system.file("extdata", "TFBSs_1.qs",
package="SingleMoleculeFootprinting"))
SortedReads = SortReadsByTFCluster(MethSM = Methylation[[2]], TFBS_cluster = TFBSs)
StateQuantification(SortedReads = SortedReads, states = TFPairStates())

```

StateQuantificationBySingleTF

Convenience for calculating state frequencies after sorting reads by single TF

Description

wraps around StateQuantification function

Usage

```
StateQuantificationBySingleTF(SortedReads)
```

Arguments

SortedReads List of sorted reads (can be multiple samples) as returned by SortReadsBySingleTF (or SortReads run with analogous parameters)

Value

tibble with state frequency information

Examples

```
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",  
package="SingleMoleculeFootprinting"))  
TFBSs = qs::qread(system.file("extdata", "TFBSs_3.qs",  
package="SingleMoleculeFootprinting"))  
SortedReads = SortReadsBySingleTF(MethSM = Methylation[[2]], TFBS = TFBSs)  
StateQuantificationBySingleTF(SortedReads = SortedReads)
```

StateQuantificationByTFPair

Convenience for calculating state frequencies after sorting reads by TF pair

Description

wraps around StateQuantification function

Usage

```
StateQuantificationByTFPair(SortedReads)
```

Arguments

SortedReads List of sorted reads (can be multiple samples) as returned by SortReadsByTF-Cluster run for clusters of size 2 (or SortReads run with analogous parameters)

Value

tibble with state frequency information

Examples

```
Methylation = qs::qread(system.file("extdata", "Methylation_4.qs",
package="SingleMoleculeFootprinting"))
TFBSs = qs::qread(system.file("extdata", "TFBSs_1.qs",
package="SingleMoleculeFootprinting"))
SortedReads = SortReadsByTFCluster(MethSM = Methylation[[2]], TFBS_cluster = TFBSs)
StateQuantificationByTFPair(SortedReads = SortedReads)
```

StateQuantificationPlot

Plot states quantification bar

Description

Plot states quantification bar

Usage

```
StateQuantificationPlot(SortedReads, states)
```

Arguments

SortedReads Sorted reads object as returned by SortReads function
states either SingleTFStates() or TFPairStates()

Value

Bar plot quantifying states

Examples

```
library(GenomicRanges)

RegionOfInterest = GRanges("chr12", IRanges(20464551, 20465050))
Methylation = qs::qread(system.file("extdata", "Methylation_3.qs",
package="SingleMoleculeFootprinting"))
TFBSs = qs::qread(system.file("extdata", "TFBSs_3.qs", package="SingleMoleculeFootprinting"))
SortedReads = SortReadsBySingleTF(MethSM = Methylation[[2]], TFBS = TFBSs)
```

```
StateQuantificationPlot(SortedReads = SortedReads, states = SingleTFStates())
```

SubsetGRangesForSamples

Subset Granges for given samples

Description

Inner utility for LowCoverageMethRateDistribution

Usage

```
SubsetGRangesForSamples(GRanges_obj, Samples)
```

Arguments

GRanges_obj	GRanges object as returned by CallContextMethylation function
Samples	vector of sample names as they appear in the SampleName field of the QuasR sampleFile

TFPairStateQuantificationPlot

TF pair state quantification bar

Description

TF pair state quantification bar

Usage

```
TFPairStateQuantificationPlot(SortedReads)
```

Arguments

SortedReads	Sorted reads as returned by SortReadsByTFCluster
-------------	--

TFPairStates	<i>Design states for TF pair case</i>
--------------	---------------------------------------

Description

Design states for TF pair case

Usage

TFPairStates()

Value

list of states

Examples

TFPairStates()

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