Package ‘SingleMoleculeFootprinting’

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Title Analysis tools for Single Molecule Footprinting (SMF) data

Version 1.12.0

Depends R (>= 4.1.0)

Imports BiocGenerics, Biostrings, BSgenome, GenomeInfoDb,
GenomicRanges, data.table, grDevices, plyr, IRanges,
RColorBrewer, stats, QuasR

Description SingleMoleculeFootprinting is an R package providing 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

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

License GPL-3

Encoding UTF-8

Suggests BSgenome.Mmusculus.UCSC.mm10, devtools, ExperimentHub, knitr,
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testthat (>= 3.0.0)

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Contents

BaitCapture *Bait capture efficiency*

Description

check bait capture efficiency. Expected to be ~70

Usage

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

Arguments

sampleSheet	QuasR sample sheet
genome	BS genome
baits	Full path to bed file containing bait coordinates. If chromosome names are in e.g. "1" format, they'll be temporarily converted to "chr1"
clObj	cluster object to emply for parallel processing created using the parallel::makeCluster function. Defaults to NULL

Value

bait capture efficiency

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    # DO NOT RUN
    # clObj = parallel::makeCluster(5)
    # BaitRegions = SingleMoleculeFootprintingData::EnrichmentRegions_mm10.rds()
   # BaitCaptureEfficiency = BaitCapture(sampleSheet = Qinput, genome = BSgenome.Mmusculus.UCSC.mm10, baits = Bait
    # parallel::stopCluster(clObj)
}
```

BinMethylation *Summarize methylation inside sorting bins*

Description

Summarize methylation inside sorting bins

Usage

```
BinMethylation(MethSM, TFBS, bin)
```

Arguments

MethSM	Single molecule matrix
TFBS	Transcription factor binding site to use for sorting, passed as a GRanges object of length 1
bin	vector of two integers representing the coordinate of a bin relative to the center of the TFBS

Value

Reads covering bin with their summarized methylation status

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

   TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
    elementMetadata(TFBSs)$name = c("NRF1")
    names(TFBSs) = c(paste0("TFBS_", c(4305216)))

   binMethylationValues = BinMethylation(MethSM = Methylation[[2]], TFBS = TFBSs, bin = c(-15,15))
}
```

CallContextMethylation

Call Context Methylation

Description

Can deal with multiple samples

Usage

```
CallContextMethylation(
  sampleSheet,
  sample,
  genome,
  range,
  coverage = 20,
  ConvRate.thr = 0.2,
  verbose = TRUE
)
```

Arguments

sampleSheet	QuasR pointer file
sample	for now this works for sure on one sample at the time only
genome	BSgenome
range	GenimocRange representing the genomic region of interest
coverage	coverage threshold. Defaults to 20.
ConvRate.thr	Convesion rate threshold. Double between 0 and 1, defaults to 0.2
verbose	TRUE/FALSE

Value

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

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")
```

```
    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)
}
```

CollapseStrands *Collapse strands*

Description

Collapse strands

Usage

```
CollapseStrands(MethGR, context, verbose = TRUE)
```

Arguments

MethGR	Granges obj of average methylation
context	"GC" or "CG". Broad because indicates just the directionality of collapse.
verbose	TRUE/FALSE

Value

MethGR with collapsed strands (everything turned to - strand)

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, verbose = TRUE)
```

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
verbose	TRUE/FALSE

Value

Strand collapsed MethSM

ConversionRate *Conversion rate*

Description

calculate sequencing library conversion rate on a chromosome of choice

Usage

```
ConversionRate(sampleSheet, genome, chr = 19, clObj = NULL)
```

Arguments

sampleSheet	QuasR sample sheet
genome	BS genome
chr	chromosome to calculate conversion rate on (default: 19)
clObj	cluster object to emply for parallel processing created using the parallel::makeCluster function. Defaults to NULL

Value

Conversion rate

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    # DO NOT RUN
    # clObj = parallel::makeCluster(5)
   # ConversionRatePrecision = ConversionRate(sampleSheet = Qinput, genome = BSgenome.Mmusculus.UCSC.mm10, chr = 1
    # parallel::stopCluster(clObj)
}
```

CoverageFilter *Filter Cs for coverage*

Description

Filter Cs for coverage

Usage

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

Arguments

MethGR	Granges obj of average methylation
thr	converage threshold

Value

filtered MethGR

DetectExperimentType *Detect type of experiment*

Description

Detect type of experiment

Usage

```
DetectExperimentType(Samples, verbose = TRUE)
```

Arguments

Samples	SampleNames field from QuasR sampleSheet
verbose	TRUE/FALSE

Value

String indicating the type of experiment detected

Examples

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

if(file.exists(Qinput)){
    sample = readr::read_delim(Qinput, delim = "\t")$SampleName
    ExpType = DetectExperimentType(sample)
}
```

FilterByConversionRate

Calculate reads conversion rate

Description

Calculate reads conversion rate

Usage

```
FilterByConversionRate(MethSM, chr, genome, thr = 0.2, verbose = TRUE)
```

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 above which to filter reads. Defaults to 0.2
verbose	TRUE/FALSE

Value

Filtered MethSM

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    sample = readr::read_delim(Qinput, delim = "\t")$SampleName
   range = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    MethSM = GetSingleMolMethMat(QuasRprj, range, sample)
   MethSM = FilterByConversionRate(MethSM, chr = "chr6", 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

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    Samples = readr::read_delim(Qinput, delim = "\t")$SampleName
    sample = Samples[1]
   range = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

   MethGR = QuasR::qMeth(QuasRprj[grep(sample, Samples)], mode="allC", range, collapseBySample = TRUE, keepZero =
    FilterContextCytosines(MethGR, BSgenome.Mmusculus.UCSC.mm10, "NGCNN")
}
```

FixOverhang — *Fixing overhang before stand collapsing*

Description

Fixing overhang before stand collapsing

Usage

```
FixOverhang(MethGR, context, which)
```

Arguments

MethGR	Granges obj of average methylation
context	context
which	"Top"|"Bottom"

Value

MethGR with fixed overhang

GetQuasRprj — *Get QuasRprj*

Description

Get QuasRprj

Usage

```
GetQuasRprj(sampleSheet, genome)
```

Arguments

sampleSheet	QuasR pointer file
genome	BSgenome

Value

QuasR project object as returned by QuasR::qAlign function

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)
}
```

GetSingleMolMethMat *Get Single Molecule methylation matrix*

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 qAling 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 pointer file provided to qAlign. N.b. all the files with the passed sample name will be used to call methylation

Value

Single molecule methylation matrix (all Cytosines)

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    sample = readr::read_delim(Qinput, delim = "\t")$SampleName
   range = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    MethSM = GetSingleMolMethMat(QuasRprj, range, sample)
}
```

HierarchicalClustering *Perform Hierarchical clustering on single reads*

Description

Perform Hierarchical clustering on single reads

Usage

```
HierarchicalClustering(MethSM)
```

Arguments

MethSM	Single molecule methylation matrix

Value

Single molecule matrix after hierarchical clustering

OneTFstates *Design states for single TF case*

Description

Design states for single TF case

Usage

```
OneTFstates()
```

Value

list of states

PlotAvgSMF *Plot average methylation*

Description

Plot average methylation

Usage

```
PlotAvgSMF(MethGR, range, TFBSs)
```

Arguments

MethGR	Average methylation GRanges obj
range	GRanges interval to plot
TFBSs	GRanges object of transcription factor binding sites to include in the plot. Assumed to be already subset.

Value

Average SMF signal at single site

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

   TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
    elementMetadata(TFBSs)$name = c("NRF1")
    names(TFBSs) = c(paste0("TFBS_", c(4305216)))

    PlotAvgSMF(MethGR = Methylation[[1]], range = Region_of_interest, TFBSs = TFBSs)
}
```

PlotSingleMoleculeStack *Plot single molecule stack*

Description

Plot single molecule stack

Usage

```
PlotSingleMoleculeStack(MethSM, range)
```

Arguments

MethSM	Single molecule methylation matrix
range	GRanges interval to plot

Value

Single molecule plot

PlotSingleSiteSMF *Plot SMF data at single site*

Description

Plot SMF data at single site

Usage

```
PlotSingleSiteSMF(
  ContextMethylation,
  sample,
  range,
  SortedReads = NULL,
  TFBSs,
  saveAs = NULL
)
```

Arguments

ContextMethylation	Context methylation object as returned by CallContextMethylation function
sample	one sample as reported in the SampleName files of the QuasR sampleSheet
range	GRange interval to plot
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
TFBSs	GRange or GRangesList of transcription factor binding sites to add to the plot. If SortedReads are passed, the format of TFBSs (GRanges vs GRangesList) will be used to determie if single molecules were sorted based on one or multiple TFs
saveAs	Full path to pdf file to save plot to. Defaults to NULL, in which case will only display

Value

Single site plot including average SMF signal, single molecules stack and state quantification plot

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

   TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
    elementMetadata(TFBSs)$name = c("NRF1")
    names(TFBSs) = c(paste0("TFBS_", c(4305216)))
    SortedReads = SortReadsByTFCluster(MethSM = Methylation[[2]], TFBSs = TFBSs)

    PlotSingleSiteSMF(ContextMethylation = Methylation,
                      sample = MySample,
                      range = Region_of_interest,
                      SortedReads = SortedReads,
                      TFBSs = TFBSs,
                      saveAs = NULL)
}
```

PlotSM *Wrapper for PlotSingleMoleculeStack function*

Description

adds the convenience of arranging reads before plotting

Usage

```
PlotSM(MethSM, range, SortedReads = NULL)
```

Arguments

MethSM	Single molecule methylation matrix
range	GRanges interval to plot
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

Value

Single molecule stack plot

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

    PlotSM(MethSM = Methylation[[2]], range = Region_of_interest)
}
```

SampleCorrelation *Intersample correlation*

Description

pair plot of sample correlations

Usage

```
SampleCorrelation(samples, context, CellType)
```

Arguments

samples	Avg methylation object. Can also be set to "Example" to produce plot using example data of the kind specified by the @param CellType
context	one of "AllCs", "DGCHN", "NWCGW". The first should be chosen for TKO experiments. For experiments carried on WT cells, we recommend checking both the "DGCHN" and "NWCGW" contexts by running this function once per context.
CellType	Cell type to compare your samples to. At the moment, this can be one of "ES", "NP", "TKO".

Value

Inter-sample correlation plot

SingleTFStateQuantificationPlot *Single TF state quantification bar*

Description

Single TF state quantification bar

Usage

```
SingleTFStateQuantificationPlot(states, OrderedReads)
```

Arguments

states	as returned by OneTFstates function
OrderedReads	Reads ordered by states

Value

single TF state quantification plot

SortReads *Sort reads by single TF*

Description

Sort reads by single TF

Usage

```
SortReads(MethSM, TFBS, BinsCoord, SortByCluster)
```

Arguments

MethSM	Single molecule matrix
TFBS	Transcription factor binding site to use for sorting
BinsCoord	list of 3 bin coordinates relative to the center of the TFBS.
SortByCluster	T/F

Value

list of sorted reads

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

   TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
    elementMetadata(TFBSs)$name = c("NRF1")
    names(TFBSs) = c(paste0("TFBS_", c(4305216)))
    BinsCoord = list(c(-35,-25), c(-15,15), c(25,35))

    SortedReads = SortReads(Methylation[[2]], TFBSs, BinsCoord, SortByCluster = FALSE)
}
```

SortReadsBySingleTF *Wrapper to SortReads for single TF case*

Description

Wrapper to SortReads for single TF case

Usage

```
SortReadsBySingleTF(MethSM, TFBS)
```

Arguments

MethSM	Single molecule matrix
TFBS	Transcription factor binding site to use for sorting, passed as a GRanges object of length 1

Value

List of reads sorted by single TF

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

   TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
    elementMetadata(TFBSs)$name = c("NRF1")
    names(TFBSs) = c(paste0("TFBS_", c(4305216)))

    SortedReads = SortReadsBySingleTF(MethSM = Methylation[[2]], TFBS = TFBSs)
}
```

SortReadsByTFCluster *Wrapper to SortReads for TF cluster case*

Description

Wrapper to SortReads for TF cluster case

Usage

```
SortReadsByTFCluster(MethSM, TFBSs)
```

Arguments

MethSM	Single molecule matrix
TFBSs	Transcription factor binding sites to use for sorting, passed as a GRanges object of length > 1

Value

List of reads sorted by TF cluster

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

   TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
    elementMetadata(TFBSs)$name = c("NRF1")
    names(TFBSs) = c(paste0("TFBS_", c(4305216)))

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

StateQuantificationPlot

Plot states quantification bar

Description

Plot states quantification bar

Usage

```
StateQuantificationPlot(SortedReads)
```

Arguments

SortedReads	Sorted reads object as returned by SortReads function

Value

Bar plot quantifying states

Examples

```
Qinput = paste0(tempdir(), "/NRF1Pair_Qinput.txt")
library(BSgenome.Mmusculus.UCSC.mm10)

if(file.exists(Qinput)){
    QuasRprj = GetQuasRprj(Qinput, BSgenome.Mmusculus.UCSC.mm10)

    MySample = readr::read_delim(Qinput, delim = "\t")$SampleName[1]
   Region_of_interest = GRanges(seqnames = "chr6", ranges = IRanges(start = 88106000, end = 88106500), strand = "*")

    Methylation = CallContextMethylation(sampleSheet = Qinput,
                                         sample = MySample,
                                         genome = BSgenome.Mmusculus.UCSC.mm10,
                                         range = Region_of_interest,
                                         coverage = 20,
                                         ConvRate.thr = 0.2)

   TFBSs = GenomicRanges::GRanges("chr6", IRanges(c(88106253), c(88106263)), strand = "-")
    elementMetadata(TFBSs)$name = c("NRF1")
    names(TFBSs) = c(paste0("TFBS_", c(4305216)))

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

TFPairStateQuantificationPlot *TF pair state quantification bar*

Description

TF pair state quantification bar

Usage

```
TFPairStateQuantificationPlot(states, OrderedReads)
```

Arguments

`states`	as returned by TFpairStates function
`OrderedReads`	Reads ordered by states

Value

TF pair state quantification plot

TFpairStates *Design states for TF pair case*

Description

Design states for TF pair case

Usage

```
TFpairStates()
```

Value

list of states

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