# 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, parallel, rmarkdown, readr, SingleMoleculeFootprintingData, 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

# Index