Package ‘VALERIE’

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Title Visualising Splicing at Single-Cell Resolution

Version 1.1.0

Description Alternative splicing produces a variety of different protein products from a given gene. 'VALERIE' enables visualisation of alternative splicing events from high-throughput single-cell RNA-sequencing experiments. 'VALERIE' computes percent spliced-in (PSI) values for user-specified genomic coordinates corresponding to alternative splicing events. PSI is the proportion of sequencing reads supporting the included exon/intron as defined by Shiozawa (2018) <doi:10.1038/s41467-018-06063-x>. PSI are inferred from sequencing reads data based on specialised infrastructures for representing and computing annotated genomic ranges by Lawrence (2013) <doi:10.1371/journal.pcbi.1003118>. Computed PSI for each single cell are subsequently presented in the form of a heatmap implemented using the 'pheatmap' package by Kolde (2010) <https://CRAN.R-project.org/package=pheatmap>. Board overview of the mean PSI difference and associated p-values across different user-defined groups of single cells are presented in the form of a line graph using the 'ggplot2' package by Wickham (2007) <https://CRAN.R-project.org/package=ggplot2>.

Imports GenomicAlignments (>= 1.16.0), GenomicRanges (>= 1.32.0), IRanges (>= 2.14.0), Rsamtools (>= 1.34.0), plyr (>= 1.8.4), ggplot2 (>= 3.0.0), pheatmap (>= 1.0.10), ggplotify (>= 0.0.3), ggpubr (>= 0.2.4), scales (>= 1.0.0)

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`ComputePSI` *Percent spliced-in (PSI) computation*

Description

`ComputePSI` computes percent spliced-in (PSI) at each genomic coordinate for exon-level alternative splicing events, namely skipped exon (SE), mutually exclusive exons (MXE), retained intron (RI), alternative 5' splice site (A5SS), and alternative 3' splice site (A3SS)

Usage

```
ComputePSI(SampleInfo, ExonInfo, BAM, MinCoverage)
```

Arguments

`SampleInfo`	Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.
`ExonInfo`	Tab-delimited file describing the alternative splicing events. First columns contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2018) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, and A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.
`BAM`	Folder containing the BAM files sorted by genomic coordinates.
`MinCoverage`	numeric. Coverage (Total reads) threshold below which the PSI value of the genomic coordinate is annotate as missing value, i.e. no coverage.

Details

This function computes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons. Formula for computing PSI is number of reads with non-N CIGAR operation divided by the total number of reads. Total number of reads is the sum of reads with non-N CIGAR operation and reads with N-CIGAR operation

Value

A data frame of class rehab where rows are the genomic coordinates and columns are the sample names.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- ComputePSI(SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_small.txt", package="VALERIE"),
  ExonInfo=system.file("extdata/Exon_Info", "Exon_Info.txt", package="VALERIE"),
  BAM=system.file("extdata/BAM", "", package="VALERIE"),
  MinCoverage=10)
PSI[1:5,1:4]
```

PlotPSI — *Percent spliced-in (PSI) visualization for alternative splicing events*

Description

`PlotPSI` visualizes percent spliced-in (PSI) for each genomic coordinate for alternative splicing events across two groups of single cells.

Usage

```
PlotPSI(object, SampleInfo, ExonInfo, statistical.test = c("wilcox",
  "t.test", "KW", "ANOVA"), multiple.testing = c("holm", "hochberg",
  "hommel", "bonferroni", "BH", "BY", "fdr", "none"), Plots, plot.width,
  plot.height, EventType = c("SE", "MXE", "RI", "A5SS", "A3SS"), Groups)
```

Arguments

`object`	Object of class rehab generated using `ComputePSI`.
`SampleInfo`	Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1, Group2, etc. Third column indicates the group names. Example file provided in extdata directory of the package.

`ExonInfo` Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, and A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.

`statistical.test` Method for comparising PSI values at each genomic coordinates between groups of single cells. Parametric methods include student t-test and analysis of variance. Non-parametric methods include wilcoxon rank sum test and Kruskal-Wallis test.

`multiple.testing` Method for adjusting p-values for multiple comparisons.

`Plots` Folder to output PSI plots.

`plot.width` Width of outplot plots.

`plot.height` Height of outplot plots.

`EventType` Indicates the type of alternative splicing event to plot.

`Groups` Indicate the number of groups of single cells.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric (student t-test/ANOVA) or non-parameteric (Wilcoxon rank-sum/Kruskal-Wallis) test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for the two groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between the groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Two_Groups_small.rds", package="VALERIE"))
PlotPSI(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Two_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
```

```
  statistical.test="wilcox",  multiple.testing="fdr",
  Plots=tempdir(),
  plot.width=5, plot.height=8, EventType="SE", Groups=2)
```

PlotPSI.A3SS.MultiGroups

Percent spliced in (PSI) visualization for alternative 3' splice sites (A3SS)

Description

PlotPSI.A3SS visualizes percent spliced-in (PSI) for each genomic coordinate for alternative 3' split site (A3SS) event across groups of single cells.

Usage

```
PlotPSI.A3SS.MultiGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("KW", "ANOVA"), multiple.testing = c("holm",
  "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none"), Plots,
  plot.width, plot.height)
```

Arguments

object	Object of class rehab generated using ComputePSI.
SampleInfo	Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.
ExonInfo	Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.
statistical.test	Method for comparising PSI values at each genomic coordinates between groups of single cells.
multiple.testing	Method for adjusting p-values for multiple comparisons.
Plots	Folder to output PSI plots.
plot.width	Width of outplot plots.
plot.height	Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric ANOVA or non-parameteric Kruskal-Wallis test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Multi_Groups_small.rds", package="VALERIE"))
PlotPSI.A3SS.MultiGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Multi_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="KW",  multiple.testing="bonferroni",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

PlotPSI.A3SS.TwoGroups

Percent spliced in (PSI) visualization for alternative 3' splice sites (A3SS)

Description

`PlotPSI.A3SS` visualizes percent spliced-in (PSI) for each genomic coordinate for alternative 3' split site (A3SS) event across two groups of single cells.

Usage

```
PlotPSI.A3SS.TwoGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("wilcox", "t.test"),
  multiple.testing = c("holm", "hochberg", "hommel", "bonferroni", "BH",
  "BY", "fdr", "none"), Plots, plot.width, plot.height)
```

Arguments

`object` Object of class rehab generated using `ComputePSI`.

`SampleInfo` Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.

`ExonInfo` Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.

`statistical.test` Method for comparising PSI values at each genomic coordinates between two groups of single cells.

`multiple.testing` Method for adjusting p-values for multiple comparisons.

`Plots` Folder to output PSI plots.

`plot.width` Width of outplot plots.

`plot.height` Height of outplot plots.

Details

This function visualizes the percent spliced in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line group to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric student t-test or non-parameteric Wilcoxon rank-sum test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for two groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between two groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Two_Groups_small.rds", package="VALERIE"))
PlotPSI.A3SS.TwoGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Two_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="wilcox",  multiple.testing="bonferroni",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

PlotPSI.A5SS.MultiGroups

Percent spliced-in (PSI) visualization for alternative 5' splice sites (A5SS)

Description

`PlotPSI.A5SS` visualizes percent spliced-in (PSI) for each genomic coordinate for alternative 5' splice site (A5SS) event across groups of single cells.

Usage

```
PlotPSI.A5SS.MultiGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("KW", "ANOVA"), multiple.testing = c("holm",
  "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none"), Plots,
  plot.width, plot.height)
```

Arguments

`object`	Object of class rehab generated using `ComputePSI`.
`SampleInfo`	Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.
`ExonInfo`	Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.
`statistical.test`	Method for comparising PSI values at each genomic coordinates between groups of single cells.
`multiple.testing`	Method for adjusting p-values for multiple comparisons.
`Plots`	Folder to output PSI plots.
`plot.width`	Width of outplot plots.
`plot.height`	Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric ANOVA or non-parameteric Kruskal-Wallis test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Multi_Groups_small.rds", package="VALERIE"))
PlotPSI.A5SS.MultiGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Multi_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="KW",  multiple.testing="none",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

PlotPSI.A5SS.TwoGroups

Percent spliced-in (PSI) visualization for alternative 5' splice sites (A5SS)

Description

`PlotPSI.A5SS` visualizes percent spliced-in (PSI) for each genomic coordinate for alternative 5' splice site (A5SS) event across two groups of single cells.

Usage

```
PlotPSI.A5SS.TwoGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("wilcox", "t.test"),
  multiple.testing = c("holm", "hochberg", "hommel", "bonferroni", "BH",
  "BY", "fdr", "none"), Plots, plot.width, plot.height)
```

Arguments

`object` Object of class rehab generated using `ComputePSI`.

`SampleInfo` Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.

`ExonInfo` Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.

`statistical.test` Method for comparising PSI values at each genomic coordinates between two groups of single cells.

`multiple.testing` Method for adjusting p-values for multiple comparisons.

`Plots` Folder to output PSI plots.

`plot.width` Width of outplot plots.

`plot.height` Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric student t-test or non-parameteric Wilcoxon rank-sum test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for two groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between two groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Two_Groups_small.rds", package="VALERIE"))
PlotPSI.A5SS.TwoGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Two_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="wilcox",  multiple.testing="fdr",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

PlotPSI.MXE.MultiGroups

Percent spliced-in (PSI) visualization for mutually exclusive exons (MXE)

Description

`PlotPSI.MXE` visualizes percent spliced-in (PSI) for each genomic coordinate for mutually exclusive exons (MXE) event across groups of single cells.

Usage

```
PlotPSI.MXE.MultiGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("KW", "ANOVA"), multiple.testing = c("holm",
  "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none"), Plots,
  plot.width, plot.height)
```

Arguments

`object`	Object of class rehab generated using `ComputePSI`.
`SampleInfo`	Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.
`ExonInfo`	Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.
`statistical.test`	Method for comparising PSI values at each genomic coordinates between groups of single cells.
`multiple.testing`	Method for adjusting p-values for multiple comparisons.
`Plots`	Folder to output PSI plots.
`plot.width`	Width of outplot plots.
`plot.height`	Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric ANOVA or non-parameteric Kruskal-Wallis test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Multi_Groups_small.rds", package="VALERIE"))
PlotPSI.MXE.MultiGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Multi_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="KW",  multiple.testing="bonferroni",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

`PlotPSI.MXE.TwoGroups` *Percent spliced-in (PSI) visualization for mutually exclusive exons (MXE)*

Description

`PlotPSI.MXE` visualizes percent spliced-in (PSI) for each genomic coordinate for mutually exclusive exons (MXE) event across two groups of single cells.

Usage

```
PlotPSI.MXE.TwoGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("wilcox", "t.test"),
  multiple.testing = c("holm", "hochberg", "hommel", "bonferroni", "BH",
  "BY", "fdr", "none"), Plots, plot.width, plot.height)
```

Arguments

`object` Object of class rehab generated using `ComputePSI`.

`SampleInfo` Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.

`ExonInfo` Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.

`statistical.test` Method for comparising PSI values at each genomic coordinates between two groups of single cells.

`multiple.testing` Method for adjusting p-values for multiple comparisons.

`Plots` Folder to output PSI plots.

`plot.width` Width of outplot plots.

`plot.height` Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric student t-test or non-parameteric Wilcoxon rank-sum test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for two groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between two groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Two_Groups_small.rds", package="VALERIE"))
PlotPSI.MXE.TwoGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Two_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="wilcox",  multiple.testing="fdr",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

PlotPSI.RI.MultiGroups

Percent spliced-in (PSI) visualization for retained introns (RI)

Description

`PlotPSI.RI` visualizes percent spliced-in (PSI) for each genomic coordinate for retained intron (RI) event across groups of single cells.

Usage

```
PlotPSI.RI.MultiGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("KW", "ANOVA"), multiple.testing = c("holm",
  "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none"), Plots,
  plot.width, plot.height)
```

Arguments

`object`	Object of class rehab generated using `ComputePSI`.
`SampleInfo`	Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.
`ExonInfo`	Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.
`statistical.test`	Method for comparising PSI values at each genomic coordinates between groups of single cells.
`multiple.testing`	Method for adjusting p-values for multiple comparisons.
`Plots`	Folder to output PSI plots.
`plot.width`	Width of outplot plots.
`plot.height`	Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric ANOVA or non-parameteric Kruskal-Wallis test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Multi_Groups_small.rds", package="VALERIE"))
PlotPSI.RI.MultiGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Multi_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="KW",  multiple.testing="none",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

`PlotPSI.RI.TwoGroups` *Percent spliced-in (PSI) visualization for retained introns (RI)*

Description

`PlotPSI.RI` visualizes percent spliced-in (PSI) for each genomic coordinate for retained intron (RI) event across two groups of single cells.

Usage

```
PlotPSI.RI.TwoGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("wilcox", "t.test"),
  multiple.testing = c("holm", "hochberg", "hommel", "bonferroni", "BH",
  "BY", "fdr", "none"), Plots, plot.width, plot.height)
```

Arguments

`object` Object of class rehab generated using `ComputePSI`.

`SampleInfo` Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.

`ExonInfo` Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.

`statistical.test` Method for comparising PSI values at each genomic coordinates between two groups of single cells.

`multiple.testing` Method for adjusting p-values for multiple comparisons.

`Plots` Folder to output PSI plots.

`plot.width` Width of outplot plots.

`plot.height` Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric student t-test or non-parameteric Wilcoxon rank-sum test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for two groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between two groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Two_Groups_small.rds", package="VALERIE"))
PlotPSI.RI.TwoGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Two_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="t.test",  multiple.testing="none",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

PlotPSI.SE.MultiGroups

Percent spliced-in (PSI) visualization for skipped exons (SE)

Description

`PlotPSI.SE` visualizes percent spliced-in (PSI) for each genomic coordinate for skipped exon (SE) event across groups of single cells.

Usage

```
PlotPSI.SE.MultiGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("KW", "ANOVA"), multiple.testing = c("holm",
  "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none"), Plots,
  plot.width, plot.height)
```

Arguments

`object`	Object of class rehab generated using `ComputePSI`.
`SampleInfo`	Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1, Group2, etc. Third column indicates the group names. Example file provided in extdata directory of the package.
`ExonInfo`	Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, and A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.
`statistical.test`	Method for comparising PSI values at each genomic coordinates between more groups of single cells.
`multiple.testing`	Method for adjusting p-values for multiple comparisons.
`Plots`	Folder to output PSI plots.
`plot.width`	Width of outplot plots.
`plot.height`	Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric ANOVA or non-parameteric Kruskal-Wallis test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for the groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Multi_Groups_small.rds", package="VALERIE"))
PlotPSI.SE.MultiGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Multi_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="KW",  multiple.testing="bonferroni",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

`PlotPSI.SE.TwoGroups` *Percent spliced-in (PSI) visualization for skipped exons (SE)*

Description

`PlotPSI.SE` visualizes percent spliced-in (PSI) for each genomic coordinate for skipped exon (SE) event across two groups of single cells.

Usage

```
PlotPSI.SE.TwoGroups(object, SampleInfo, ExonInfo,
  statistical.test = c("wilcox", "t.test"),
  multiple.testing = c("holm", "hochberg", "hommel", "bonferroni", "BH",
  "BY", "fdr", "none"), Plots, plot.width, plot.height)
```

Arguments

`object` Object of class rehab generated using `ComputePSI`.

`SampleInfo` Tab-delimited file describing the naming and grouping of the single cells. First column should contain the names of the binary alignment map (BAM) files. Second column indicates the grouping for each single cell, namely Group1 and Group2. Third column indicates the group names. Example file provided in extdata directory of the package.

`ExonInfo` Tab-delimited file describing the alternative splicing events. First column contains the alternative splicing nomenclature as per BRIE (Huang et al, Genome Biology, 2019) or MISO (Katz et al, Nature Methods, 2010). Second column indicates the type of alternative splicing event, namely SE, MXE, RI, A5SS, and A3SS. Third column contains the gene name or any personal notation. Example file provided in extdata directory of the package.

`statistical.test` Method for comparising PSI values at each genomic coordinates between two groups of single cells.

`multiple.testing` Method for adjusting p-values for multiple comparisons.

`Plots` Folder to output PSI plots.

`plot.width` Width of outplot plots.

`plot.height` Height of outplot plots.

Details

This function visualizes the percent spliced-in (PSI) at each genomic coordinate encompassing the alternative exon and its flanking constitutive exons for each single cell in the form of a heatmap. The PSI mean for the respective groups are also display in the form of a line graph to summarize the PSI distributions of the respective groups. Pair-wise comparison of PSI at each genomic coordinate is performed using either the parametric student t-test or non-parameteric Wilcoxon rank-sum test. The p-values can be adjusted for multiple testing using the `p.adjust` function.

Value

For each alternative splicing event, a single plot consisting of three subplots arranged from top to bottom is returned. Bottom subplot is a line graph of PSI means at each genomic coordinate for two groups of single cells. Middle subplot is a line graph of p-values corresponding to the comparison of PSI values at each genomic coordinate between two groups of single cells. Top subplot is a heatmap of PSI values at each genomic coordinate across all single cells. Location of plots as per specified in the `Plots` argument.

Author(s)

Sean Wen <sean.wenwx@gmail.com>

Examples

```
PSI <- readRDS(system.file("extdata/PSI", "PSI_RED_Two_Groups_small.rds", package="VALERIE"))
PlotPSI.SE.TwoGroups(PSI, SampleInfo=system.file("extdata/Sample_Info",
  "Sample_Info_RED_Two_Groups.txt", package="VALERIE"),
 ExonInfo=system.file("extdata/Exon_Info", "Exon_Info_RED_small.txt", package="VALERIE"),
  statistical.test="wilcox",  multiple.testing="fdr",
  Plots=tempdir(), plot.width=5, plot.height=8)
```

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