Package 'PICB'

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Title piRNA Cluster Builder

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Description piRNAs (short for PIWI-
interacting RNAs) and their PIWI protein partners play a key role in fertility
and maintaining genome integrity by restricting mobile genetic elements (transposons) in germ cells.
piRNAs originate from genomic regions known as piRNA clusters. The piRNA Cluster Builder (PICB) is
a versatile toolkit designed to identify genomic regions with a high density of piRNAs. It constructs
piRNA clusters through a stepwise integration of unique and multimapping piRNAs and offers wide-ranging
parameter settings, supported by an optimization function that allows users to test different parameter
combinations to tailor the analysis to their specific piRNA system. The output includes extensive metadata
columns, enabling researchers to rank clusters and extract cluster characteristics.

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`PICB-package` *PICB: piRNA Cluster Builder*

Description

piRNAs (short for PIWI-interacting RNAs) and their PIWI protein partners play a key role in fertility and maintaining genome integrity by restricting mobile genetic elements (transposons) in germ cells. piRNAs originate from genomic regions known as piRNA clusters. The piRNA Cluster Builder (PICB) is a versatile toolkit designed to identify genomic regions with a high density of piRNAs. It constructs piRNA clusters through a stepwise integration of unique and multimapping piRNAs and offers wide-ranging parameter settings, supported by an optimization function that allows users to test different parameter combinations to tailor the analysis to their specific piRNA system. The output includes extensive metadata columns, enabling researchers to rank clusters and extract cluster characteristics.

Main Functions

The package provides several core functions:

- `PICBload`: Load and preprocess BAM files containing piRNA alignments
- `PICBbuild`: Build piRNA seeds/cores/clusters from alignments
- `PICBoptimize`: Optimize parameters for cluster building
- `PICBstrandanalysis`: Add sense/antisense ratio of unique piRNAs per piRNAcluster
- `PICBannotate`: Annotate GRanges according to a piRNA library
- `PICBloadfasta`: Get SeqInfo object from a fasta file
- `PICBexporttoexcel`: Export cluster object into an Excel file
- `PICBimporttoexcel`: Import cluster object from an Excel file

Workflow

A typical PICB workflow consists of:

1. Loading alignments with `PICBload`
2. Building clusters with `PICBbuild`
3. Optional parameter optimization with `PICBoptimize`
4. Optional strand analysis with `PICBstrandanalysis`
5. Exporting results with `PICBexporttoexcel`

Author(s)

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See Also

Useful links:

- https://github.com/HaaseLab/PICB
- Report bugs at https://github.com/HaaseLab/PICB/issues

Examples

```
# 0. Load PICB
library(PICB)

# 1. Load Required Genome from e.g. Seqinfo (check all options in Vignette or ReadMe)
myGenome <- Seqinfo::Seqinfo(
    seqnames = c("chr2L", "chr2R", "chr3L", "chr3R", "chr4", "chrX", "chrY"),
    seqlengths = c(23513712, 25286936, 28110227, 32079331, 1348131, 23542271, 3667352)
)

# 2. Load Example Data and Process Alignments
bam_file <- system.file("extdata", "Fly_Ov1_chr2L_20To21mb_filtered.bam",
    package = "PICB")

myAlignments <- PICBload(
    BAMFILE = bam_file,
    REFERENCE.GENOME = myGenome,
    VERBOSE = FALSE
)

# 3. Build piRNA Clusters
myClusters <- PICBbuild(
    IN.ALIGNMENTS = myAlignments,
    REFERENCE.GENOME = myGenome,
    LIBRARY.SIZE = 12799826,  # Usually calculated automatically
    VERBOSITY = 0
)$clusters

# 4. Optimize Parameters (Optional)
parameterExploration <- PICBoptimize(
    IN.ALIGNMENTS = myAlignments,
    REFERENCE.GENOME = myGenome,
    MIN.UNIQUE.ALIGNMENTS.PER.WINDOW = c(1, 2, 3, 4, 5),
    LIBRARY.SIZE = 12799826,  # Usually calculated automatically
    VERBOSITY = 1
)

# 5. Perform Strand Analysis
myClustersWithStrand <- PICBstrandanalysis(
    IN.ALIGNMENTS = myAlignments,
    IN.RANGES = myClusters
)

# 6. Export Clusters
PICBexporttoexcel(
    IN.RANGES = myClustersWithStrand,
    EXCEL.FILE.NAME = "myClusters_demonstration.xlsx"
)
# 7. Import Ranges
importedClusters <- PICBimportfromexcel(
   EXCEL.FILE.NAME = system.file("extdata", "myClusters_demonstration.xlsx", package = "PICB")
)
```

PICBannotate *Annotate GRanges according to a piRNA library*

Description

Annotate GRanges according to a piRNA library

Usage

```
PICBannotate(
  INPUT.GRANGES,
  ALIGNMENTS,
  REFERENCE.GENOME = NULL,
  REPLICATE.NAME = NULL,
  LIBRARY.SIZE = length(ALIGNMENTS$unique) + length(ALIGNMENTS$multi.primary),
  PROVIDE.NON.NORMALIZED = FALSE,
  SEQ.LEVELS.STYLE = "UCSC",
  COMPUTE.1U.10A.FRACTIONS = FALSE
)
```

Arguments

INPUT.GRANGES	GRanges (seeds/cores/clusters) to annotate
ALIGNMENTS	list of alignments from PICBload
REFERENCE.GENOME	name of genome. For example "BSgenome.Dmelanogaster.UCSC.dm6"
REPLICATE.NAME	name of the replicate. NULL by default.
LIBRARY.SIZE	number of reads in the library. By default computed as number of unique mapping alignments + number of primary multimapping alignments.
PROVIDE.NON.NORMALIZED	provide annotations in non-normalized format. False by default.
SEQ.LEVELS.STYLE	naming of chromosomes style. "UCSC" by default.
COMPUTE.1U.10A.FRACTIONS	for each locus and each alignments type (unique mapping, primary multimapping, secodnary multimapping) compute fraction 1U and 10A containing reads overlapping the locus. Default FALSE.

Value

GRanges object with extra annotation columns

Author(s)

Aleksandr Friman

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)
myGenome <- "BSgenome.Dmelanogaster.UCSC.dm6"
myAlignmentsFromPICBload <- PICBload(
   BAMFILE = system.file("extdata", "Fly_Ov1_chr2L_20To21mb_filtered.bam", package = "PICB"),
    REFERENCE.GENOME = myGenome,
    VERBOSE = FALSE
)
myRangesFromPICBbuild <- PICBbuild(
    IN.ALIGNMENTS = myAlignmentsFromPICBload,
    REFERENCE.GENOME = myGenome,
    VERBOSITY = 0
)

myClustersFromPICBbuildAnnotationsRemoved <- GenomicRanges::granges(myRangesFromPICBbuild$clusters)

PICBannotate(
    INPUT.GRANGES = myClustersFromPICBbuildAnnotationsRemoved,
    ALIGNMENTS = myAlignmentsFromPICBload,
    REFERENCE.GENOME = myGenome,
    PROVIDE.NON.NORMALIZED = TRUE
)
```

PICBbuild *Build piRNA seeds/cores/clusters from alignments*

Description

Build piRNA seeds/cores/clusters from alignments

Usage

```
PICBbuild(
  IN.ALIGNMENTS,
  REFERENCE.GENOME,
  UNIQUEMAPPERS.SLIDING.WINDOW.WIDTH = 350,
 UNIQUEMAPPERS.SLIDING.WINDOW.STEP = round(UNIQUEMAPPERS.SLIDING.WINDOW.WIDTH/10, 0),
  PRIMARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH = 350,
  PRIMARY.MULTIMAPPERS.SLIDING.WINDOW.STEP =
    round(PRIMARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH/10, 0),
  SECONDARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH = 1000,
  SECONDARY.MULTIMAPPERS.SLIDING.WINDOW.STEP =
    round(SECONDARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH/10, 0),
 LIBRARY.SIZE = length(IN.ALIGNMENTS$unique) + length(IN.ALIGNMENTS$multi.primary),
 MIN.UNIQUE.ALIGNMENTS.PER.WINDOW = 2 * (UNIQUEMAPPERS.SLIDING.WINDOW.WIDTH/1000) *
    (LIBRARY.SIZE/1e+06),
  MIN.UNIQUE.SEQUENCES.PER.WINDOW = min(MIN.UNIQUE.ALIGNMENTS.PER.WINDOW,
    round(UNIQUEMAPPERS.SLIDING.WINDOW.WIDTH/50, 0)),
```

```
  MIN.PRIMARY.MULTIMAPPING.ALIGNMENTS.PER.WINDOW = 4 *
    (PRIMARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH/1000) * (LIBRARY.SIZE/1e+06),
  MIN.SECONDARY.MULTIMAPPING.ALIGNMENTS.PER.WINDOW = 0.2 *
    (SECONDARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH/1000) * (LIBRARY.SIZE/1e+06),
  MIN.SEED.LENGTH = 2 * UNIQUEMAPPERS.SLIDING.WINDOW.WIDTH + 100,
  MIN.COVERED.SEED.LENGTH = 0,
  THRESHOLD.SEEDS.GAP = 0,
  THRESHOLD.CORES.GAP = 0,
  THRESHOLD.CLUSTERS.GAP = 0,
  SEQ.LEVELS.STYLE = "UCSC",
  MIN.OVERLAP = 5,
  PROVIDE.NON.NORMALIZED = FALSE,
  COMPUTE.1U.10A.FRACTIONS = FALSE,
  VERBOSITY = 2
)
```

Arguments

IN.ALIGNMENTS	list of alignments from PICBload
REFERENCE.GENOME	name of genome. For example "BSgenome.Dmelanogaster.UCSC.dm6"
UNIQUEMAPPERS.SLIDING.WINDOW.WIDTH	width of sliding window for unique mappers. 350 nt by default
UNIQUEMAPPERS.SLIDING.WINDOW.STEP	step of sliding windows for unique mappers. width/10 by default
PRIMARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH	width of sliding window for primary multimapping alignments. 350 nt by default
PRIMARY.MULTIMAPPERS.SLIDING.WINDOW.STEP	step of sliding windows for primary multimapping alignments. width/10 by default
SECONDARY.MULTIMAPPERS.SLIDING.WINDOW.WIDTH	width of sliding window for secondary multimapping alignments. 1000 nt by default
SECONDARY.MULTIMAPPERS.SLIDING.WINDOW.STEP	step of sliding windows for secondary multimapping alignments. width/10 by default
LIBRARY.SIZE	number of reads in the library. By default computed as number of unique mapping alignments + number of primary multimapping alignments.
MIN.UNIQUE.ALIGNMENTS.PER.WINDOW	absolute number of unique mapping alignments per window to call it. By default computed as 2 FPKM.
MIN.UNIQUE.SEQUENCES.PER.WINDOW	absolute number of unique mapping sequences per window to call it. By default computed as width/50.
MIN.PRIMARY.MULTIMAPPING.ALIGNMENTS.PER.WINDOW	absolute number of primary multimapping alignments per window to call it. By default computed as 4 FPKM.

MIN.SECONDARY.MULTIMAPPING.ALIGNMENTS.PER.WINDOW
: absolute number of secondary multimapping alignments per window to call it. By default computed as 0.2 FPKM.

MIN.SEED.LENGTH
: minimum length of a seed. By default computed as 2x unique mapper window size + 100.

MIN.COVERED.SEED.LENGTH
: minimum number of seed nucleotides covered by unique mappers. 0 by default.

THRESHOLD.SEEDS.GAP
: minimum gap between seeds to not merge them. 0 by default.

THRESHOLD.CORES.GAP
: minimum gap between cores to not merge them. 0 by default.

THRESHOLD.CLUSTERS.GAP
: minimum gap between clusters to not merge them. 0 by default.

SEQ.LEVELS.STYLE
: naming of chromosomes style. "UCSC" by default.

MIN.OVERLAP
: minimum overlap between seeds and cores, as well as between cores and clusters 5 nt by default.

PROVIDE.NON.NORMALIZED
: include non-normalized to the library size statistics in the output annotations

COMPUTE.1U.10A.FRACTIONS
: for each locus and each alignments type (unique mapping, primary multimapping, secodnary multimapping) compute fraction 1U and 10A containing reads overlapping the locus. Default FALSE.

VERBOSITY
: verbosity level 0/1/2/3. 2 by default.

Value

list of annotated GRanges objects named "seeds" for seeds, "cores" for cores, "clusters" for clusters

Author(s)

Pavol Genzor

Daniel Stoyko

Aleksandr Friman

Franziska Ahrend

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)
myAlignmentsFromPICBload <- PICBload(
   BAMFILE = system.file("extdata", "Fly_Ov1_chr2L_20To21mb_filtered.bam", package = "PICB"),
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    VERBOSE = FALSE
)

outputOfPICBbuild <- PICBbuild(
```

```
    IN.ALIGNMENTS = myAlignmentsFromPICBload,
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    VERBOSITY = 0
)
```

PICBexporttoexcel *Export cluster object into an Excel file*

Description

Export cluster object into an Excel file

Usage

```
PICBexporttoexcel(IN.RANGES = NULL, EXCEL.FILE.NAME = NULL)
```

Arguments

IN.RANGES	clustering object to export
EXCEL.FILE.NAME	file name to save

Value

no values returned

Author(s)

Aleksandr Friman

Franziska Ahrend

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)
myAlignmentsFromPICBload <- PICBload(
   BAMFILE = system.file("extdata", "Fly_Ov1_chr2L_20To21mb_filtered.bam", package = "PICB"),
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    VERBOSE = FALSE
)

outputOfPICBbuild <- PICBbuild(
    IN.ALIGNMENTS = myAlignmentsFromPICBload,
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    LIBRARY.SIZE = 12799826, #usually not necessary
    VERBOSITY = 0
)
PICBexporttoexcel(
    IN.RANGES = outputOfPICBbuild,
    EXCEL.FILE.NAME = "inst/extdata/myClusters_demonstration.xlsx"
)
```

PICBgetchromosomes *Get SeqInfo object from standard non-circular chromosome names from your genome*

Description

Get SeqInfo object from standard non-circular chromosome names from your genome

Usage

```
PICBgetchromosomes(REFERENCE.GENOME, SEQ.LEVELS.STYLE = "UCSC")
```

Arguments

REFERENCE.GENOME	name of genome. For example "BSgenome.Dmelanogaster.UCSC.dm6", or directly a SeqInfo object.
SEQ.LEVELS.STYLE	naming of chromosomes style. "UCSC" by default.

Value

SeqInfo object with standard non-circular chromosome names

Author(s)

Aleksandr Friman

Franziska Ahrend

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)
mySI <- PICBgetchromosomes("BSgenome.Dmelanogaster.UCSC.dm6", "UCSC")
```

PICBimportfromexcel *Import cluster object from an Excel file*

Description

Import cluster object from an Excel file

Usage

```
PICBimportfromexcel(EXCEL.FILE.NAME = NULL)
```

Arguments

EXCEL.FILE.NAME
: file name to import from

Value

list of annotated GRanges objects named "seeds" for seeds, "cores" for cores, "clusters" for clusters

Author(s)

Aleksandr Friman

Examples

```
importedClusters <- PICBimportfromexcel(
   EXCEL.FILE.NAME = system.file("extdata", "myClusters_demonstration.xlsx", package = "PICB")
)
```

PICBload *Load and preprocess BAM files containing piRNA alignments*

Description

Load and preprocess BAM files containing piRNA alignments

Usage

```
PICBload(
  BAMFILE = NULL,
  REFERENCE.GENOME = NULL,
  SIMPLE.CIGAR = TRUE,
  IS.SECONDARY.ALIGNMENT = NA,
  STANDARD.CONTIGS.ONLY = TRUE,
  PERFECT.MATCH.ONLY = FALSE,
  FILTER.BY.FLAG = TRUE,
  SELECT.FLAG = c(0, 16, 272, 256),
  USE.SIZE.FILTER = TRUE,
  READ.SIZE.RANGE = c(18, 50),
  TAGS = c("NH", "NM"),
  WHAT = c("flag"),
  SEQ.LEVELS.STYLE = "UCSC",
  GET.ORIGINAL.SEQUENCE = FALSE,
  VERBOSE = TRUE
)
```

Arguments

BAMFILE	name of the bam file to load. Should be sorted and indexed.
REFERENCE.GENOME	name of genome. For example "BSgenome.Dmelanogaster.UCSC.dm6"
SIMPLE.CIGAR	simpleCigar parameter of Rsamtools::ScanBamParam
IS.SECONDARY.ALIGNMENT	defines loading of primary/secondary alignments. Default value NA loads both primary and secondary.
STANDARD.CONTIGS.ONLY	use only standard chromosomes
PERFECT.MATCH.ONLY	load only alignments without mismatches
FILTER.BY.FLAG	enables filtering by flag. TRUE by default.
SELECT.FLAG	vector of flags to use. Default value c(0,16, 272, 256).
USE.SIZE.FILTER	enables filter by alignment size. True by default.
READ.SIZE.RANGE	allowed alignment sizes. c(18,50) by default.
TAGS	tags to import from bam file. c("NH","NM") by default.
WHAT	"what" parameter of Rsamtools::ScanBamParam. c("flag") by default.
SEQ.LEVELS.STYLE	naming of chromosomes style. "UCSC" by default.
GET.ORIGINAL.SEQUENCE	adds "seq" to WHAT. False by default.
VERBOSE	enables progress output. True by default.

Value

list of GRanges objects named "unique" for unique mapping alignments, "multi.primary" for primary multimapping alignments, "multi.secondary" for secondary multimapping alignments

Author(s)

Pavol Genzor

Daniel Stoyko

Aleksandr Friman

Franziska Ahrend

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)
PICBload(
   BAMFILE = system.file("extdata", "Fly_Ov1_chr2L_20To21mb_filtered.bam", package = "PICB"),
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    VERBOSE = FALSE
)
```

PICBloadfasta *Get SeqInfo object from a fasta file*

Description

Get SeqInfo object from a fasta file

Usage

```
PICBloadfasta(FASTA.NAME = NULL)
```

Arguments

FASTA.NAME	path to the fasta file

Value

SeqInfo object with all chromosome names and lengths from the fasta file

Author(s)

Aleksandr Friman

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)

# create temporary fasta file
chr2L_seq <- BSgenome.Dmelanogaster.UCSC.dm6[["chr2L"]]
chr2L_seq_set <- DNAStringSet(chr2L_seq)
names(chr2L_seq_set) <- "chr2L"
temp_fasta <- tempfile(fileext = ".fasta")
writeXStringSet(chr2L_seq_set, temp_fasta)

myGenome <- PICBloadfasta(FASTA.NAME = temp_fasta)
unlink(temp_fasta)
```

PICBoptimize *Runs PICBbuild multiple times with provided parameters and returns optimization data frame.*

Description

Runs PICBbuild multiple times with provided parameters and returns optimization data frame.

Usage

```
PICBoptimize(
  IN.ALIGNMENTS,
  REFERENCE.GENOME,
 LIBRARY.SIZE = length(IN.ALIGNMENTS$unique) + length(IN.ALIGNMENTS$multi.primary),
  VERBOSITY = 2,
  PROVIDE.INFO.SEEDS.AND.CORES = FALSE,
  SEQ.LEVELS.STYLE = "UCSC",
  ...
)
```

Arguments

IN.ALIGNMENTS	list of alignments from PICBload
REFERENCE.GENOME	name of genome. For example "BSgenome.Dmelanogaster.UCSC.dm6"
LIBRARY.SIZE	number of reads in the library. By default computed as number of unique mapping alignments + number of primary multimapping alignments.
VERBOSITY	verbosity level 0/1/2/3. 2 by default.
PROVIDE.INFO.SEEDS.AND.CORES	FALSE by default.
SEQ.LEVELS.STYLE	naming of chromosomes style. "UCSC" by default.
...	rest of the parameters used by PICBbuild and provided as iteratable vectors

Value

Optimization values dataframe

Author(s)

Aleksandr Friman

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)
myAlignmentsFromPICBload <- PICBload(
   BAMFILE = system.file("extdata", "Fly_Ov1_chr2L_20To21mb_filtered.bam", package = "PICB"),
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    VERBOSE = FALSE
)

PICBoptimize(
    IN.ALIGNMENTS = myAlignmentsFromPICBload,
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    MIN.UNIQUE.ALIGNMENTS.PER.WINDOW = c(1, 2, 3, 4, 5)
)
```

PICBstrandanalysis *Add sense/antisense ratio of unique piRNAs per piRNA cluster*

Description

Add sense/antisense ratio of unique piRNAs per piRNA cluster

Usage

```
PICBstrandanalysis(IN.ALIGNMENTS, IN.RANGES, VERBOSE = TRUE)
```

Arguments

IN.ALIGNMENTS	list of alignments from PICBload
IN.RANGES	single GRanges object (seeds, cores or clusters from PICBbuild)
VERBOSE	enables progress output. True by default.

Value

GRanges object with an additional annotation column

Author(s)

Parthena Konstantinidou

Zuzana Loubalova

Franziska Ahrend

Examples

```
library(BSgenome.Dmelanogaster.UCSC.dm6)
myAlignmentsFromPICBload <- PICBload(
   BAMFILE = system.file("extdata", "Fly_Ov1_chr2L_20To21mb_filtered.bam", package = "PICB"),
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    VERBOSE = FALSE
)

outputOfPICBbuild <- PICBbuild(
    IN.ALIGNMENTS = myAlignmentsFromPICBload,
    REFERENCE.GENOME = "BSgenome.Dmelanogaster.UCSC.dm6",
    VERBOSITY = 0
)

outputOfPICBbuild$clusters <- PICBstrandanalysis(
    IN.ALIGNMENTS = myAlignmentsFromPICBload,
    IN.RANGES = outputOfPICBbuild$clusters
)
```

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