

Package ‘PICB’

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Title piRNA Cluster Builder

Version 1.5.0

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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BugReports <https://github.com/HaaseLab/PICB/issues>

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PICB-package	<i>PICB: piRNA Cluster Builder</i>
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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.

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,
                secondary 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.FRACCTIONS = 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, secondary 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	<i>Get SeqInfo object from standard non-circular chromosome names from your genome</i>
--------------------	--

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	<i>Runs PICBbuild multiple times with provided parameters and returns optimization data frame.</i>
--------------	--

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 iterable 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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