

# Package ‘PICB’

June 5, 2026

**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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**Encoding** UTF-8

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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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