Package ‘Ularcirc’

May 3, 2024

Type Package

Title Shiny app for canonical and back splicing analysis (i.e. circular and mRNA analysis)

Version 1.22.0

Description Ularcirc reads in STAR aligned splice junction files and provides visualisation and analysis tools for splicing analysis. Users can assess backsplice junctions and forward canonical junctions.

biocViews DataRepresentation, Visualization, Genetics, Sequencing, Annotation, Coverage, AlternativeSplicing, DifferentialSplicing

License file LICENSE

Encoding UTF-8

LazyData true

Depends R (>= 3.4.0)

Imports AnnotationHub, AnnotationDbi, BiocGenerics, Biostrings, BSgenome, data.table (>= 1.9.4), DT, GenomicFeatures, GenomeInfoDb, GenomeInfoDbData, GenomicAlignments, GenomicRanges, ggplot2, ggrepel, gsubfn, mirbase.db, moments, Organism.dplyr, plotgardener, R.utils, S4Vectors, shiny, shinydashboard, shinyFiles, shinyjs, yaml

RoxygenNote 7.1.2

Suggests BSgenome.Hsapiens.UCSC.hg38, BioCStyle, httpuv, knitr, org.Hs.eg.db, rmarkdown, TxDb.Hsapiens.UCSC.hg38.knownGene

VignetteBuilder knitr

git_url https://git.bioconductor.org/packages/Ularcirc

git_branch RELEASE_3_19

git_last_commit 839155f

git_last_commit_date 2024-04-30

Repository Bioconductor 3.19

Date/Publication 2024-05-02

Author David Humphreys [aut, cre]

Maintainer David Humphreys <d.humphreys@victorchang.edu.au>
BSJ_details

This function returns details of a BSJ string and returns a list of coordinates. Can accept two different formats, Ularcirc or generic.

Usage

BSJ_details(BSJ)

Arguments

BSJ : backsplice junction as a string. See details below for example formats

Examples

bsj <- 'chr14_99465814_chr14_99458278'  # Historic Ularcirc format

bsj <- c("chr14_99465814_chr14_99458278","chr22_20933778_chr22_20934245","chr12_120155720_chr12_120154969","chr4_143543508_chr4_143543973","chr10_7285955_chr10_7276891")

BSJ_details(bsj)

bsj <- 'chr10:100923974-100926020:+'  # generic format
Description
Takes a circRNA predicted sequence and generates synthetic short sequence reads

Usage
```
bsj_fastq_generate(
    circRNA_Sequence,
    fragmentLength = 300,
    readLength = 100,
    variations = 4,
    headerID = ""
)
```

Arguments
- `circRNA_Sequence` : Linear sequence of a circRNA. i.e. the backsplice junction is the first and last base of this sequence
- `fragmentLength` : Is the length the library fragment
- `readLength` : The sequence read length
- `variations` : Number of sequences returned for each read type. Note each sequence variation will start at a unique location (where possible)
- `headerID` : Character identifier that will be incorporated into sequence header

Value
Returns a list of two DNAstring sets labelled "read1" and "read2" which correspond to forward and reverse read pairs.

Examples
```
library('Ularcirc')

# Generate a 500nt sequence containing A and which is flanked with GG and CC.
circRNA_Sequence <- paste(rep('A',500),collapse='')
circRNA_Sequence <- paste('GG',circRNA_Sequence, 'CC', sep='')
# The GG and CC ends of sequence represent ends of linear exons that are circularised.
# Therefore the backsplice junction (BSJ) is GGCC.
# Generate reads that alternate over this BSJ
```
bsj_to_circRNA_sequence

Description
Takes one BSJ coordinate and generates a predicted circular RNA sequence.

Usage

```r
bsj_to_circRNA_sequence(
  BSJ,
  geneID = NULL,
  genome,
  TxDb,
  annotationLibrary,
  reduce_candidates = TRUE,
  shiny = FALSE
)
```

Arguments

- **BSJ**: BSJ coordinate in the format of chr_coordinate_chr_coordinate:strand.
- **geneID**: The gene ID that the BSJ aligns to. Not essential as this can be identified from the BSJ coordinate, however time performance of function improved if this information can be provided.
- **genome**: Is the length f the library fragment
- **TxDb**: The sequence read length
- **annotationLibrary**: annotation database. See details for example.
bsj_to_circRNA_sequence

reduce_candidates
: IF multiple exon entries align to a single BSJ then either return longest entry (TRUE) or all entries (FALSE)

shiny
: If TRUE then will setup shiny progress bars. Default is FALSE where a standard text progress bar is used.

Details
Backsplice junction coordinates are typically reported as a character string. Two formats are recognised, ":" delimited (eg circExplorer, CIRI) or "_" delimited (Ularcirc). The BSJ genomic coordinates are compared against the supplied gene model and exonic sequences from matching splice junctions are concatenated. This means the BSJ is the first and last nucleotide of the returned sequence. The current implementation will automatically check 0 or 1 base coordinates and any match is returned.

In some cases one BSJ will match multiple exon combinations. The default setting is to return the longest sequence. Alternatively all possibilities can be returned by setting reduce_candidates to FALSE. BSJ candidates that align to multiple exon combinations are added to duplicated list. BSJ that do not align to any canonical junctions are returned as failed.

Value
Returns a DNAstring object.

Examples

library('Ularcirc')
TxDb <- TxDb.Hsapiens.UCSC.hg38.knownGene::TxDb.Hsapiens.UCSC.hg38.knownGene
genome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
annotationLibrary <- org.Hs.eg.db::org.Hs.eg.db

# Define BSJ. Following two formats are accepted
BSJ <- 'chr2:40430305-40428472:' 
# SLC8A1
BSJ <- 'chr2_40430305_chr2_40428472' 
# SLC8A1

circRNA_sequence <- bsj_to_circRNA_sequence(BSJ, "SLC8A1", genome, TxDb, annotationLibrary)

# You can also retrieve sequence without passing gene annotation - but this is slower
# circRNA_sequence <- bsj_to_circRNA_sequence(BSJ, NULL, genome, TxDb, annotationLibrary)

TxDb <- TxDb.Hsapiens.UCSC.hg38.knownGene::TxDb.Hsapiens.UCSC.hg38.knownGene
genome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38

# EXAMPLE1 (3 fail and 2 will produce sequences)
BSJ <- c("chr14_99465814_chr14_99458278", "chr22_20933778_chr22_20934245", "chr12_120155720_chr12_120154969", "chr4_143543508_chr4_143543973", "chr10_7285955_chr10_7276891")
GeneIDs <- c("SMARCA5","MSLN","RNFL38","KIAA0368","CRKL")
circRNA_sequence <- bsj_to_circRNA_sequence(BSJ, GeneIDs, genome, TxDb, annotationLibrary)

# Returns a list with three items:
# (1) "identified" is a list of DNA strings from BSJ that aligned to FSJ coordinates of the gene model
# (2) "failed" is a character object of BSJ that did not align to FSJ coordinates of gene model. Each entry is
chimericFilters

# named with gene ID.
# (3) "duplicates" (not implemented yet) identifies which BSJ returned multiple sequences

chimericFilters  chimericFilters

Description

A wrapper function that prepares a list of filters that can be passed

Usage

chimericFilters(
  BSjuncName = NULL,
  sortDir = "Descending",
  indexNumber = 1,
  displayNumber = 10,
  displayRADscore = FALSE,
  RADcountThreshold = 10,
  applyFSJfilter = FALSE
)

Arguments

  BSjuncName  : A character string that represents a backsplice junction ID. Set when needing
to extract a specific junction. Default NULL.
  sortDir     : Specifies how data is sorted, either "Descending" (default) or "Ascending".
  indexNumber : Filter data according to this file index
  displayNumber : Number of records to display in an shiny app
  displayRADscore : Boolean. If TRUE then will apply/calculate RAD score
  RADcountThreshold : Integer. The minimum count threshold required to calculate RAD score. i.e.
                      A default RAD score of -1 will be applied to any BSJ with a count less than this
                      score
  applyFSJfilter : Boolean of whether to apply FSJ filter
### chimericStats

**Description**

Simple function that returns a list of basic stats obtained from a STAR chimeric file.

**Usage**

chimericStats(chimericDT)

**Arguments**

- `chimericDT`: Data table of chimeric junctions as provided by STAR aligner.

**See Also**

FilterChimericJuncs

**Examples**

```r
extdata_path <- system.file("extdata", package = "Ularcirc")
chimeric.file <- paste0(extdata_path, "/SRR444655_subset.Chimeric.out.junction.gz")
chimericDT <- Ularcirc::loadSTAR_chimeric(chimeric.file, returnColIdx = 1:14)
Ularcirc::chimericStats(chimericDT$data_set)
chimericDT$filtered <- Ularcirc::FilterChimericJuncs(chimericDT$data_set, canonicalJuncs = TRUE)
Ularcirc::chimericStats(chimericDT$filtered)
```

---

### Compatible_Annotation_DBs

**Description**

Interogates Bioconductor databases and identifies those that are compatible with Ularcirc. Builds a list of commands that the user can copy to install the required database on their local computer. Once installed the databases are immediately available to Ularcirc upon re-starting the shiny app. This function requires connection to the internet.

**Usage**

Compatible_Annotation_DBs(search_term = "")
Arguments

search_term : character string of a full or part name of a database. Will return only those entries that contain this search term. Not case sensitive.

Value

Returns a list of compatible annotation databases

Examples

# Get all Bioconductor annotation databases that are compatible with Ularcirc
library('BSgenome')
library('httpuv')
library('AnnotationHub')
# Prepare a dataframe of all compatible annotation databases
## Not run: compatible_DBs_human <- Compatible_Annotation_DBs("Hsapiens")

# Example of how to find a relevant database and load the relevant databases:
# This example find hg38 databases
idx <- grep(pattern="hg38", x= compatible_DBs_human[,"genome"])
if (!requireNamespace("BiocManager", quietly=TRUE))
  install.packages("BiocManager")
BiocManager::install(c(compatible_DBs[idx,]))

## End(Not run)

FilterChimericJuncs

Description

A generic function that filters STAR chimeric junction files on certain genomic criteria (eg strand, same chromosome etc). Useful filter to remove the most obvious false positives. The default filter settings are suitable for circRNA discovery in humans / mice data sets.

Usage

FilterChimericJuncs(
  All_junctions,
  chromFilter = TRUE,
  strandFilter = TRUE,
  genomicDistance = c(200, 1e+05),
  canonicalJuncs = TRUE,
  fileID = c(-1),
  chrM_Filter = TRUE,
  invertReads = FALSE
)
FilterChimeric_Ularcirc

Arguments

All_junctions : data.table of chimeric reads from STAR aligner
chromFilter : when TRUE (default) both chimera parts have to align to same chromosome
strandFilter : when TRUE (default) both chimera parts have to align to same strand
genomicDistance : minimum and maximum distance filters of chimeric reads on chromosome. Only is applied if ChromFilter is TRUE and StrandFilter is TRUE
canonicalJuncs : Will include any canonical junctions (default TRUE). Note STAR keeps canonical junctions that do not conform to aligner rules.
fileID : Specify a file index. Useful if planning to concatenating all data sets into a single table.
chrM_Filter : Filter out mitochondrial chimeric reads (default TRUE)
invertReads : Boolean that specifies in read strand should be inverted (default FALSE).

See Also

SelectUniqueJunctions, loadSTAR_chimeric

Examples

```r
extdata_path <- system.file("extdata",package = "Ularcirc")
chimeric.file <- paste0(extdata_path,"/SRR444655_subset.Chimeric.out.junction.gz")
chimericsDT <- Ularcirc::loadSTAR_chimeric(chimeric.file,returnColIdx = 1:14)
chimericsDT$filtered <- Ularcirc::FilterChimericJuncs(chimericsDT$data_set, canonicalJuncs = TRUE)
```

FilterChimeric_Ularcirc

Wrapper function for Ularcirc shiny app which expects a list of objects to be returned

Description

NEED to ensure that unstranded boolean value is passed to this function Not tested via shiny app yet.

Usage

```r
FilterChimeric_Ularcirc(
  All_junctions,
  chromFilter = TRUE,
  strandFilter = TRUE,
  genomicDistance = c(200, 1e+05),
  canonicalJuncs = TRUE,
  fileID = c(-1),
)```
Junction_Sequence_from_Genome

chrM_Filter = TRUE,
invertReads = FALSE,
unstranded = FALSE,
summaryNumber = 50

Arguments

All_junctions : data.table of chimeric reads from STAR aligner
chromFilter : when TRUE (default) both chimera parts have to align to same chromosome
strandFilter : when TRUE (default) both chimera parts have to align to same strand
genomicDistance : minimum and maximum distance filters of chimeric reads on chromosome. Only is applied if ChromFilter is TRUE and StrandFilter is TRUE
canonicalJuncs : Will include any canonical junctions (default TRUE). Note STAR keeps canonical junctions that do not conform to aligner rules.
fileID : Specify a file index. Useful if planning to concatenating all data sets into a single table.
chrM_Filter : Filter out mitochondrial chimeric reads (default TRUE)
invertReads : Boolean that specifies in read strand should be inverted (default FALSE).
unstranded : Boolean for if reads are unstranded
summaryNumber : Number (Integer) of records to display in shiny app

Junction_Sequence_from_Genome

Grab_BS_Junc_Sequence

Description

This function extracts genomic sequence that is likely to capture BSJ. Function does not cross validate to gene models.

Usage

Junction_Sequence_from_Genome(SelectUniqueJunct_Value, GeneList)

Arguments

GeneList : GeneList
SelectUniqueJunct_value : a dataframe with columns names startDonor, strandDonor, startAcceptor
loadSTAR_chimeric

Description

Loads chimeric output file from the STAR aligner and returns a list containing three items (a data table, alignment stats and command line).

Usage

loadSTAR_chimeric(filename = NULL, ID_index = 0, returnColIdx = 1:21)

Arguments

filename : filename of the STAR chimeric output file. Can be gzipped
ID_index : An index (single integer) which will be added as a separate column in the returned data table. Useful when collating multiple files into one large matrix like object.
returnColIdx : Numeric index of columns to return. Default 1:15

Details

Reads in a text or gzipped chimeric output file generated by the STAR aligner. Function automatically detects if the last two lines contains meta-data (produced from STAR 2.7) onwards.

Returns a list of containing three items: (1) data_set (2) alignmentStats and (3) commandLine.

The column names of data_set are defined as c("chromDonor","startDonor","strandDonor", "chromAcceptor", "startAcceptor", "strandAcceptor", "JuncType", "RepeatLength_L", "RepeatLength_R", "ReadName", "FirstBase_1stSeq","CIGAR_1stSeg", "FirstBase_2ndSeq","CIGAR_2ndSeg", "Multimapping")

If ID_index is set to a value greater than 0 then an additional column called "DataSet" is created.

Columns can be subsetted by defining returnColIdx with an integer value that correspond to order of column names listed above.

plot_AllJunctions

Description

Plots a BSJ, FSJ and transcripts for a nominated gene. Output is combined onto a single page. This function effectively wraps plotting functions from plotgardener.
plot_AllJunctions

Usage

plot_AllJunctions(
  assembly = "hg38",
  chrom,  
  chromstart, 
  chromend, 
  BSJData,
  BSJ_colors = "black",
  FSJData,
  FSJ_colors = "black",
  geneSymbol 
)

Arguments

assembly : Genome assembly
chrom : chromosome
chromstart : Starting position of chromosome
chromend : End position of chromosome
BSJData : Backsplice junction data table
BSJ_colors : Backsplice junction assigned colours
FSJData : Forward junction data table
FSJ_colors : Forward junction assigned colours
geneSymbol : Gene symbol

Value

Returns a list of two DNAstring sets labelled "read1" and "read2" which correspond to forward and reverse read pairs.

Examples

library('Ularcirc')
# BSJ
BSJ_data <- data.table::data.table(chrom1="chr2", 
  start1=c(40139400, 40160764, 40428472, 40428472),
  end1=c(40139400, 40160764, 40428472, 40428472),
  chrom2="chr2", start2=c(40178494,40178494,40430302,40430305),
  end2=c(40178494,40178494,40430302,40430305),
  score=c(13,20,360,1751))

# FSJ
FSJstarts1 <- c(40115630,40139677,40160865,40164985,40170350,40174721,
  40174843,40175282,40278771,40430302,40430305)
FSJstarts2 <- c(40139400,40160764,40164853,40170280,40174705,40174824,
  40175260,40178386,40428472,40453160,40512348)
FSJ_data <- data.table::data.table(chrom1="chr2", start1=FSJstarts1, end1=FSJstarts1,
RAD_score

```
chrom2="chr2", start2=FSJstarts2, end2=FSJstarts2,
score=c(225,825,685,666,633,596,517,542,685,101,171))

plot_AllJunctions(assembly="hg38", chrom="chr2",
chromstart=40096769, chromend=40611554,
BSJData=BSJ_data, FSJData=FSJ_data, geneSymbol="SLC8A1")
```

**RAD_score**

<table>
<thead>
<tr>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretically the position of backsplice junctions should be distributed randomly across an amplicon. This function calculates the read alignment distribution (RAD) of backsplice junctions between forward and reverse read pairs. The RAD score is calculated from CIGAR strings which can be used to identify type II and type III alignments.</td>
</tr>
</tbody>
</table>

**Usage**

```
RAD_score(  
  CIGAR_1stSeg = NULL,
  CIGAR_2ndSeg = NULL,
  RADcountThreshold = 10,
  digits = 2
)
```

**Arguments**

- **CIGAR_1stSeg**: CIGAR string of the first segment.
- **CIGAR_2ndSeg**: CIGAR string of the second segment
- **RADcountThreshold**: Minimum count threshold required to apply RAD score. If there are less than this many entries in CIGAR list then -1 is returned.
- **digits**: rounding of the RAD score to this many digits (default 2)

**SelectUniqueJunctions**

**Description**

Builds a summary table from chimeric data obtained from the STAR aligner. Assembles table with the requested number of top entries. Populates with RAD score and FSJ score.
Usage

SelectUniqueJunctions(
    BSJ_junctions,
    filterlist = chimericFilters(),
    unstranded = FALSE,
    FSJ_Junctions = NULL,
    shinyapp = FALSE
)

Arguments

BSJ_junctions : Junction to display
filterlist : filterlist
unstranded : If TRUE will match reads from both strands.
FSJ_Junctions : Junction to display.
shinyapp : Boolean. If true used to setup control status bars in shiny app.

Details

This is the workhorse for collated BSJ junctions from the input data. It will return selected rows of data (annotated) that will enable enhanced browsing of raw data on the fly.

Filter options: Junction abundance. Sort

sequence_from_exon_coords

sequence_from_exon_coords

Description

sequence_from_exon_coords

Usage

sequence_from_exon_coords(genome, exon_df)

Arguments

genome : genome object
exon_df : data frame of exons. Must have column with names "chrom", "start", "stop", "strand"
Description

When the function is invoked the Ularcirc shiny app is started. The starting screen has quickstart instructions on how to use the software. Please refer to the Ularcirc vignette for a more detailed workflow.

Usage

Ularcirc()

Value

Does not return anything

Examples

# The following commands will load the shiny app either through an RStudio session or # through your internet browser

library("Ularcirc")
## Not run: Ularcirc()
Index

BSJ_details, 2
bsj_fastq_generate, 3
bsj_to_circRNA_sequence, 4

chimericFilters, 6
chimericStats, 7
Compatible_Annnotation_DBs, 7

FilterChimeric_Ularcirc, 9
FilterChimericJuncs, 8

Junction_Sequence_from_Genome, 10

loadSTAR_chimeric, 11

plot_AllJunctions, 11

RAD_score, 13

SelectUniqueJunctions, 13
sequence_from_exon_coords, 14

Ularcirc, 15