# Package 'CYCLeR'

August 19, 2021

```
Title CircRNA transcriptome assembly tool
Version 1.3
Description CYCLeR is a software package for assembly of circRNA transcripts from
      RNA-seq data. Takes a set of BSJ prediction files and RNA-seq BAM files as an input
     and outputs circRNA trascnripts as FASTA, GTF and flat annotation files. The tools
      also outputs a padded FASTA to serve as an index for transcript EM abundance estimation.
License GNU General Public License (v3)
LazyData yes
Depends R,
     Rsamtools,
     SummarizedExperiment,
      methods,
     tidyverse,
      SGSeq,
      igraph,
      DEXSeq,
     polyester
Imports AnnotationDbi,
      BiocGenerics,
      Biostrings,
     GenomicAlignments,
      GenomicFeatures,
      GenomeInfoDb,
      RUnit,
      S4Vectors,
      grDevices,
     graphics,
      igraph,
      parallel,
     rtracklayer,
      stats,
      tidyverse,
      SGSeq,
```

Type Package

2 combine.two.BSJ.tables

```
igraph,
 DEXSeq,
 polyester
Suggests BiocStyle,
 BSgenome. Hsapiens. UCSC. hg38,
 TxDb.Hsapiens.UCSC.hg38.knownGene,
 knitr,
 rmarkdown
VignetteBuilder knitr
biocViews TranscriptomeAssembly, RNASeq, Transcription
RoxygenNote 7.1.1
Encoding UTF-8
R topics documented:
  filter.bam
  make.BSJ.sg.....
  Index
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combine.two.BSJ.tables
       combine BSJs
Description
 Combine 2 BSJ tables
```

### Usage

```
combine.two.BSJ.tables(ce_bsjs, ciri_bsjs)
```

#### **Arguments**

```
ce_bsjs BSJ table 1 ciri_bsjs BSJ table 2
```

filter.bam 3

#### **Details**

Just a combination of BSJ tables to make sure we have a complete set of BSJs. The variable names do not actually matter since the all tables have the same formatting.

#### Value

Tibble object with combined filtered BSJ coordinate and number of junction spanning reads across sample.

### Author(s)

Stefan Stefanov

filter.bam

BAM file filter

### **Description**

A wrapper function for samtools use to trim the files

#### Usage

```
## S3 method for class 'bam'
filter(BSJ_gr, sample_table, samtools_prefix)
```

### **Arguments**

```
BSJ_gr a GRange of BSJ cooredinates

sample_table sample table formatted according to the manual, Must contain "sample_name"

"treatment" "file_bam" "lib_size" "read_len"; NB the values in column "treatment" can only be "control" and "enriched"

samtools_prefix

a string that corresponds to user's samtools run prefix
```

### Details

This function removes the BAM file reads that do not overlap with the BSJ loci. This significantly speeds us the feature detection and lowers the virtual memory requirements

#### Value

BAMFileList object with info on the trimmed files

#### Author(s)

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```
find.depleted.features
```

CircRNA feature selection

#### **Description**

CircRNA feature selection

#### Usage

```
find.depleted.features(circ_fc_adj, sample_table, circ_sg, test = "DEX")
```

### **Arguments**

circ\_fc\_adj count matrix corresponding to the circRNA features

sample\_table sample table formatted according to the manual, Must contain "sample\_name"

"two-two-st" "fels how" "lik size" "word how" ND the values in aclume "two-st."

"treatment" "file\_bam" "lib\_size" "read\_len"; NB the values in column "treat-

ment" can only be "control" and "enriched"

circ\_sg SGSeq object supplying feature info

test either "DEX" for DEXSeq based feature selection or "comparison" simple av-

erage comaparison

#### **Details**

This function works in 2 ways: direct comparison of average quantities or as a wrapper of DEXSeq. In case of dataset with replicates, the suggested approach is the use of DEXSeq statistical test.

### Value

vector of featureID

### Author(s)

Stefan Stefanov

make.BSJ.gr

Convert BSJ string to GRanges obejct

### **Description**

Convert BSJ string to GRanges obejct

#### Usage

```
make.BSJ.gr(BSJ_set)
```

make.BSJ.sg 5

### **Arguments**

BSJ\_set a list of BSJ ID records procudes by process.BSJs or combine.two.BSJ.tables

### **Details**

Convert BSJ string to GRanges obejct

#### Value

GRanges object indicating BSJ loci

### Author(s)

Stefan Stefanov

make.BSJ.sg

Preparation of the BSJ-specific splice graphs

### Description

Selection of the exons based on BSJ set

### Usage

```
make.BSJ.sg(circ_sg, BSJ_gr)
```

### Arguments

circ\_sg SGSeq prediction object
BSJ\_gr a GRange of BSJ coordinates

### **Details**

Selection of the exons based on BSJ set

### Value

SGSeq containing exons belonging to BSJ loci

### Author(s)

6 overlap.SG.BSJ

merge.qics

Merging 2 assemblies

### Description

Pair-wise merging 2 assemblies Pair-wise merging 2 assemblies

### Usage

```
## S3 method for class 'qics'
merge(qics1, qics2)
```

### Arguments

 $\begin{array}{ll} \text{qics1} & \text{assembly 1} \\ \text{qics2} & \text{assembly 2} \end{array}$ 

#### Value

data.frame of transcript information in flat format

#### Author(s)

Stefan Stefanov

overlap.SG.BSJ

Overlap of BSJ and a splice graph

### Description

Creates a disjointed set of exons based on a SGSeq obejct and a BSJ GRanges object

### Usage

```
overlap.SG.BSJ(sgfc_pred, BSJ_gr)
```

#### **Arguments**

 $\begin{tabular}{ll} {\tt sgfc\_pred} & {\tt sgSeq} \ prediction \ object \\ {\tt BSJ\_gr} & {\tt a} \ GRange \ of \ BSJ \ cooredinates \\ \end{tabular}$ 

#### **Details**

Creates a disjointed set of exons based on a SGSeq obejct and a BSJ GRanges object. The function keeps the SGSeq metadata

parse.files 7

### Value

SGSeq with disjoint exon bins

### Author(s)

Stefan Stefanov

parse.files

Parse BSJ input

### Description

Parse BSJ files from CIRI, CIRCexplorer2 or a TSV file

### Usage

```
parse.files(file_list, file_path, input_type)
```

### **Arguments**

file\_list list with file names

file\_path string object with file path, clould be an empty string

 $\verb|input_type| \qquad \verb|CIRI| for CIRI2 input|, \verb|CE| for CIRC explorer 2 input| and \verb|tsv| for TSV| formatted|$ 

input

#### **Details**

This processes BSJ prediction files and prepares them for the next step of the pipeline. input\_type is essential for the correct parsing of the files.

### Value

Tibble object with combined BSJ coordinate and number of junction spanning reads across sample

### Author(s)

8 process.BSJs

plotRanges2

Plot ranges

### **Description**

Plots GRanges objects

### Usage

```
plotRanges2(...)
```

#### **Details**

ggplot of multiple GRanges object. Every object is auto assigned a colour from colorblind friendly scheme

#### Value

ggplot of multiple GRanges objects

### Author(s)

Stefan Stefanov

process.BSJs

Process BSJs

### **Description**

process the BSJ table and select high confidence BSJs

### Usage

```
process.BSJs(cdf, sample_table)
```

### Arguments

cdf tibble produced by parse.files

sample\_table sample table formatted according to the manual, Must contain "sample\_name"

"treatment" "file\_bam" "lib\_size" "read\_len"; NB the values in column "treat-

ment" can only be "control" and "enriched"

file\_path string object with file path, clould be an empty string

#### **Details**

Filters BSJ based on comparison of the average CPM values of BSJs

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### Value

Tibble object with combined filtered BSJ coordinate and number of junction spanning reads across sample.

#### Author(s)

Stefan Stefanov

recount.features

Re-count of the reads per exon bin

### Description

A wrapper function for Rsubread

### Usage

```
recount.features(full_sg, sample_table)
```

### **Arguments**

```
full_sg a SGSeq obeject of exon bins

sample_table sample table formatted according to the manual, Must contain "sample_name"

"treatment" "file_bam" "lib_size" "read_len"; NB the values in column "treatment" can only be "control" and "enriched"
```

#### **Details**

This function performs requantification of the exon bins with specifically selected parameters

### Value

BAMFileList object with info on the trimmed files

#### Author(s)

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RPKM.calc

RPKM calculation for the genomic features

#### **Description**

RPKM calculation for the genomic features

### Usage

```
RPKM.calc(
   count_matrix,
   sg,
   bsj_granges,
   bs_genome,
   sample_table,
   feature_type,
   fsj_overhang = 3,
   bsj_overhang = 15,
   eff_length_correction = T,
   gc_correction = F
```

#### **Arguments**

```
count_matrix count matrix corresponding to the features
sg
                 SGSeq object supplying feature info
                 GRange of BSJ cooredinates
bsj_granges
bs genome
                 a BSGenome object used for extracting the sequences
sample_table sample table formatted according to the manual, Must contain "sample_name"
                 "treatment" "file_bam" "lib_size" "read_len"; NB the values in column "treat-
                 ment" can only be "control" and "enriched"
feature_type either "e" for exons ot "j" for junctions
fsj_overhang the FJS overhand used in the mapping a.k.a. anchor
bsj_overhang the BSJ overhand used in the chimeric detection
eff_length_correction
                 whether or not to apply effective length correction
gc_correction
                 whether or not to apply GC-content correction; requires further testing
```

#### **Details**

This function performs RPKM calculations for the exonic features. The RPKM calculation is performed based on the exact sequences for the exons. For junctions, the sequences are selected based on the exons, flanking the junction. The function takes into account the needed effective length corrections.

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### Value

BAMFileList object with info on the trimmed files

### Author(s)

Stefan Stefanov

```
transcripts.per.sample
```

Transcript assembly

### Description

Transcript assembly per sample Transcript assembly per sample based on sample name in the "sample\_table"

### Usage

```
transcripts.per.sample(i)
```

### Arguments

i name of the sample

### Value

data.frame of transcript information in flat format

### Author(s)

## **Index**

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