

Package ‘multicrispr’

June 6, 2023

Title Multi-locus multi-purpose Crispr/Cas design

Version 1.11.1

Encoding UTF-8

Description This package is for designing Crispr/Cas9 and Prime Editing experiments. It contains functions to (1) define and transform genomic targets, (2) find spacers (4) count offtarget (mis)matches, and (5) compute Doench2016/2014 targeting efficiency. Care has been taken for multicrispr to scale well towards large target sets, enabling the design of large Crispr/Cas9 libraries.

License GPL-2

LazyData true

RoxygenNote 7.1.2

Depends R (>= 4.0)

Imports assertive, BiocGenerics, Biostrings, BSgenome, CRISPRseek, data.table, GenomeInfoDb, GenomicFeatures, GenomicRanges, ggplot2, grid, karyoploteR, magrittr, methods, parallel, plyranges, Rbowtie, reticulate, rtracklayer, stats, stringi, tidyr, tidyselect, utils

Suggests AnnotationHub, BiocStyle, BSgenome.Hsapiens.UCSC.hg38, BSgenome.Mmusculus.UCSC.mm10, BSgenome.Scerevisiae.UCSC.sacCer1, ensemblDb, IRanges, knitr, magick, rmarkdown, testthat, TxDb.Mmusculus.UCSC.mm10.knownGene

VignetteBuilder knitr

biocViews CRISPR, Software

BugReports <https://github.com/loosolab/multicrispr/issues>

URL <https://github.com/loosolab/multicrispr>

git_url <https://git.bioconductor.org/packages/multicrispr>

git_branch devel

git_last_commit 53bb8e5

git_last_commit_date 2023-05-17

Date/Publication 2023-06-06

Author Aditya Bhagwat [aut, cre],
Rene Wiegandt [ctb],
Mette Bentsen [ctb],
Jens Preussner [ctb],
Michael Lawrence [ctb],
Hervé Pagès [ctb],
Johannes Graumann [sad],
Mario Looso [sad, rth]

Maintainer Aditya Bhagwat <aditya.bhagwat@uni-marburg.de>

R topics documented:

add_genome_matches	2
add_inverse_strand	4
add_seq	5
add_target_matches	6
bed_to_granges	7
char_to_granges	8
double_flank	8
extend_for_pe	10
extend_pe_to_gg	11
extract_matchranges	12
extract_subranges	13
find_gg	13
find_primespacers	14
find_spacers	16
genes_to_granges	18
gr2dt	19
has_been_indexed	19
index_genome	20
index_targets	21
plot_intervals	22
plot_karyogram	23
score_ontargets	24
up_flank	26
write_ranges	28
Index	29

add_genome_matches	<i>Add genome matches</i>
--------------------	---------------------------

Description

Add genome matches

Usage

```
add_genome_matches(
  spacers,
  bsgenome = getBSgenome(genome(spacers)[1]),
  mismatches = 2,
  pam = "NGG",
  offtargetmethod = c("bowtie", "pdict")[1],
  outdir = OUTDIR,
  indexedgenomesdir = INDEXEDGENOMESDIR,
  verbose = TRUE
)
```

Arguments

spacers	GRanges
bsgenome	BSgenome
mismatches	number
pam	string
offtargetmethod	'bowtie' or 'pdict'
outdir	bowtie output directory
indexedgenomesdir	directory with indexed genomes
verbose	TRUE (default) or FALSE

Value

GRanges

Examples

```
require(magrittr)
file <- system.file('extdata/SRF.bed', package='multicrispr')
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
targets0 <- bed_to_granges(file, 'mm10')
targets <- extend(targets0)
spacers <- find_spacers(targets, bsgenome, complement = FALSE,
                        ontargetmethod = NULL, offtargetmethod = NULL)
spacers %<>% extract(1:100)
spacers %<>% add_genome_matches(bsgenome)
```

add_inverse_strand	<i>Add inverse strand</i>
--------------------	---------------------------

Description

Add inverse strand

Usage

```
add_inverse_strand(gr, verbose = FALSE, plot = FALSE, ...)
```

Arguments

gr	GRanges-class
verbose	TRUE or FALSE (default)
plot	TRUE or FALSE (default)
...	plot_intervals arguments

Value

[GRanges-class](#)

Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                        HBB  = 'chr11:5227002:-',         # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+', # ins
                        bsgenome)
add_inverse_strand(gr, plot = TRUE)

# TFBS example
#-----
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, genome = 'mm10')
add_inverse_strand(gr)
```

add_seq	<i>Add sequence to GRanges</i>
---------	--------------------------------

Description

Add sequence to GRanges

Usage

```
add_seq(gr, bsgenome, verbose = FALSE, as.character = TRUE)
```

Arguments

gr	GRanges-class
bsgenome	BSgenome-class
verbose	TRUE or FALSE (default)
as.character	TRUE (default) or FALSE

Value

[GRanges-class](#)

Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                        HBB = 'chr11:5227002:-', # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+', # ins
                        bsgenome)
  (gr %<>% add_seq(bsgenome))

# TFBS example
#-----
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, 'mm10')
(gr %<>% add_seq(bsgenome))
```

add_target_matches	<i>Add target matches</i>
--------------------	---------------------------

Description

Add target matches

Usage

```
add_target_matches(
  spacers,
  targets,
  bsgenome,
  mismatches = 2,
  pam = "NGG",
  outdir = OUTDIR,
  verbose = TRUE
)
```

Arguments

spacers	GRanges
targets	GRanges
bsgenome	BSgenome
mismatches	number
pam	string
outdir	bowtie output directory
verbose	TRUE (default) or FALSE

Value

GRanges

Examples

```
require(magrittr)
file <- system.file('extdata/SRF.bed', package='multicrispr')
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
targets0 <- bed_to_granges(file, 'mm10')
targets <- extend(targets0)
spacers <- find_spacers(targets, bsgenome, complement = FALSE,
  ontargetmethod = NULL, offtargetmethod = NULL)
spacers %<>% add_target_matches(targets, bsgenome)
```

bed_to_granges	<i>Read bedfile into GRanges</i>
----------------	----------------------------------

Description

Read bedfile into GRanges

Usage

```
bed_to_granges(  
  bedfile,  
  genome,  
  txdb = NULL,  
  do_order = TRUE,  
  plot = TRUE,  
  verbose = TRUE  
)
```

Arguments

bedfile	file path
genome	string: UCSC genome name (e.g. 'mm10')
txdb	NULL (default) or TxDb-class (used for gene annotation)
do_order	TRUE (default) or FALSE: order on seqnames and star?
plot	TRUE (default) or FALSE: plot karyogram?
verbose	TRUE (default) or FALSE

Value

[GRanges-class](#)

See Also

[char_to_granges](#), [genes_to_granges](#)

Examples

```
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')  
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10  
(gr <- bed_to_granges(bedfile, genome='mm10'))
```

char_to_granges	<i>Convert character vector into GRanges</i>
-----------------	--

Description

Convert character vector into GRanges

Usage

char_to_granges(x, bsgenome)

Arguments

x character vector
bsgenome [BSgenome-class](#)

Value

[GRanges-class](#)

See Also

[bed_to_granges](#), [genes_to_granges](#)

Examples

```
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
x <- c(PRNP = 'chr20:4699600:+', # snp
      HBB = 'chr11:5227002:-', # snp
      HEXA = 'chr15:72346580-72346583:-', # del
      CFTR = 'chr7:117559593-117559595:+') # ins
gr <- char_to_granges(x, bsgenome)
plot_intervals(gr, facet_var = c('targetname', 'seqnames'))
```

double_flank	<i>Double flank</i>
--------------	---------------------

Description

Double flank

Usage

```
double_flank(
  gr,
  upstart = -200,
  upend = -1,
  downstart = 1,
  downend = 200,
  strandaware = TRUE,
  plot = FALSE,
  linetype_var = "set",
  ...
)
```

Arguments

<code>gr</code>	GRanges-class
<code>upstart</code>	upstream flank start in relation to start(<code>gr</code>)
<code>upend</code>	upstream flank end in relation to start(<code>gr</code>)
<code>downstart</code>	downstream flank start in relation to end(<code>gr</code>)
<code>downend</code>	downstream flank end in relation to end(<code>gr</code>)
<code>strandaware</code>	TRUE (default) or FALSE
<code>plot</code>	TRUE or FALSE (default)
<code>linetype_var</code>	gr var mapped to linetype
<code>...</code>	passed to <code>plot_intervals</code>

Value

[GRanges-class](#)

Examples

```
# Prime Editing example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                        HBB = 'chr11:5227002:-', # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+', # ins
                        bsgenome)
double_flank(gr, -10, -1, +1, +20, plot = TRUE)

# TFBS example
#-----
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, genome = 'mm10', plot = FALSE)
double_flank(gr, plot = TRUE)
```

extend_for_pe	<i>Extend ranges for prime editing</i>
---------------	--

Description

Extend target ranges to span in which to look for spacer-pam seqs

Usage

```
extend_for_pe(
  gr,
  bsgenome,
  nrt = 16,
  spacer = strrep("N", 20),
  pam = "NGG",
  plot = FALSE
)
```

Arguments

gr	GRanges-class
bsgenome	BSgenome-class
nrt	number: reverse transcription length
spacer	string: spacer pattern in extended IUPAC alphabet
pam	string: pam pattern in extended IUPAC alphabet
plot	TRUE (default) or FALSE

Details

Extend target ranges to find nearby spacers for prime editing

Value

[GRanges-class](#)

Examples

```
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c( PRNP = 'chr20:4699600:+',          # snp
                        HBB  = 'chr11:5227002:-',          # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+', # ins
                        bsgenome = bsgenome))
find_primespacers(gr, bsgenome)
(grext <- extend_for_pe(gr))
find_spacers(grext, bsgenome, complement = FALSE)
```

extend_pe_to_gg	<i>Extend prime editing target to find GG sites</i>
-----------------	---

Description

Extend prime editing target to find GG sites in accessible neighbourhood

Usage

```
extend_pe_to_gg(gr, nrt = 16, plot = FALSE)
```

Arguments

gr	target GRanges-class
nrt	n RT nucleotides (default 16, recommended 10-16)
plot	TRUE or FALSE (default)

Details

Extends each target range to the area in which to search for a prime editing GG duplet, as shown in the sketch below.

```
=====>---GG--->---GG---> ** <---GG <---GG <=====
```

Value

[GRanges-class](#)

Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                        HBB  = 'chr11:5227002:-',          # snp
                        HEXA  = 'chr15:72346580-72346583:-', # del
                        CFTR  = 'chr7:117559593-117559595:+', # ins
                        bsgenome))
extend_pe_to_gg(gr, plot = TRUE)
```

extract_matchranges *Extract matching subranges*

Description

Extract subranges that match pattern

Usage

```
extract_matchranges(gr, bsgenome, pattern, plot = FALSE)
```

Arguments

gr	GRanges-class
bsgenome	BSgenome{BSgenome-class}
pattern	string: search pattern in extended IUPAC alphabet
plot	TRUE or FALSE (default)

Value

[GRanges-class](#)

Examples

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                        HBB  = 'chr11:5227002:-',          # snp
                        HEXA  = 'chr15:72346580-72346583:-', # del
                        CFTR  = 'chr7:117559593-117559595:+', # ins
                        bsgenome))

gr %<>% extend_for_pe()
pattern <- strrep('N',20) %>% paste0('NGG')
extract_matchranges(gr, bsgenome, pattern, plot = TRUE)

# TFBS examples
#-----
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile  <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, 'mm10') %>% extend()
extract_matchranges(gr, bsgenome, pattern = strrep('N',20) %>% paste0('NGG'))
```

extract_subranges	<i>Extract subranges</i>
-------------------	--------------------------

Description

Extract subranges from a [GRanges-class](#) object

Usage

```
extract_subranges(gr, ir, plot = FALSE)
```

Arguments

gr	GRanges-class
ir	IRanges-class : subranges to be extracted
plot	TRUE or FALSE (default)

Value

[GRanges-class](#).

Examples

```
# Extract a subrange
gr <- GenomicRanges::GRanges(c(A = 'chr1:1-100:+', B = 'chr1:1-100:-'))
gr$targetname <- 'AB'
ir <- IRanges::IRanges(c(A = '1-10', A = '11-20', B = '1-10', B = '11-20'))
extract_subranges(gr, ir, plot = TRUE)

# Return empty GRanges for empty IRanges
extract_subranges(GenomicRanges::GRanges('chr1:345-456'), IRanges::IRanges())
```

find_gg	<i>Find GG</i>
---------	----------------

Description

Find GG

Usage

```
find_gg(gr)
```

Arguments

gr	GRanges-class
----	-------------------------------

Value[GRanges-class](#)**Examples**

```
# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+', # snp
                        HBB = 'chr11:5227002:-', # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+'), # ins
                      bsgenome)
gr %<>% extend_pe_to_gg(plot = TRUE) %>% add_seq(bsgenome)
find_gg(gr)
```

find_primespacers	<i>Find prime editing spacers</i>
-------------------	-----------------------------------

Description

Find prime editing spacers around target ranges

Usage

```
find_primespacers(
  gr,
  bsgenome,
  edits = get_plus_seq(bsgenome, gr),
  nprimer = 13,
  nrt = 16,
  ontargetmethod = c("Doench2014", "Doench2016")[1],
  offtargetmethod = c("bowtie", "pdict")[1],
  mismatches = 0,
  nickmatches = 2,
  indexedgenomesdir = INDEXEDGENOMESDIR,
  outdir = OUTDIR,
  verbose = TRUE,
  plot = TRUE,
  ...
)
```

Arguments

gr	GRanges-class
bsgenome	BSgenome-class

edits	character vector: desired edits on '+' strand. If named, names should be identical to those of gr
nprimer	n primer nucleotides (default 13, max 17)
nrt	n rev transcr nucleotides (default 16, recomm. 10-16)
ontargetmethod	'Doench2014' or 'Doench2016': on-target scoring method
offtargetmethod	'bowtie' or 'pdict'
mismatches	no of primespacer mismatches (default 0, to suppress offtarget analysis: -1)
nickmatches	no of nickspacer offtarget mismatches (default 2, to suppresses offtarget analysis: -1)
indexedgenomesdir	directory with indexed genomes (as created by index_genome)
outdir	directory where offtarget analysis output is written
verbose	TRUE (default) or FALSE
plot	TRUE (default) or FALSE
...	passed to plot_intervals

Details

Below the architecture of a prime editing site. Edits can be performed anywhere in the revtranscript area.

```

spacer pam -----=== primer revtranscript -----===== 1.....17....GG.....
.....CC..... -----extension-----

```

Value

[GRanges-class](#) with prime editing spacer ranges and following mcols: * crisprspacer: N20 spacers * crisprpam: NGG PAMs * crisprprimer: primer (on PAM strand) * crisprtranscript: reverse transcript (on PAM strand) * crisprextension: 3' extension of gRNA contains: reverse transcription template + primer binding site sequence can be found on non-PAM strand * crisprextrange: genomic range of crispr extension * Doench2016l4: on-target efficiency scores * off0, off1, off2: number of offtargets with 0, 1, 2 mismatches * off: total number of offtargets: off = off0 + off1 + ... * nickrange: nickspacer range * nickspacer: nickspacer sequence * nickDoench2016l4: nickspacer Doench scores * nickoff: nickspacer offtarget counts

See Also

[find_spacers](#) to find standard crispr sites

Examples

```

# Find PE spacers for 4 clinically relevant loci (Anzalone et al, 2019)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(
  PRNP = 'chr20:4699600:+',          # snp: prion disease
  HBB  = 'chr11:5227002:-',          # snp: sickle cell anemia

```

```

    HEXA = 'chr15:72346580-72346583:-', # del: tay sachs disease
    CFTR = 'chr7:117559593-117559595:+'), # ins: cystic fibrosis
    bsgenome)
  spacers <- find_primespacers(gr, bsgenome)
  spacers <- find_spacers(extend_for_pe(gr), bsgenome, complement = FALSE)

# Edit PRNP locus for resistance against prion disease (Anzalone et al, 2019)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+'), bsgenome)
find_primespacers(gr, bsgenome)
find_primespacers(gr, bsgenome, edits = 'T')

```

find_spacers

Find crispr spacers in targetranges

Description

Find crispr spacers in targetranges

Usage

```

find_spacers(
  gr,
  bsgenome,
  spacer = strrep("N", 20),
  pam = "NGG",
  complement = TRUE,
  ontargetmethod = c("Doench2014", "Doench2016")[1],
  offtargetmethod = c("bowtie", "pdict")[1],
  offtargetfilterby = character(0),
  subtract_targets = FALSE,
  mismatches = 2,
  indexedgenomesdir = INDEXEDGENOMESDIR,
  outdir = OUTDIR,
  verbose = TRUE,
  plot = TRUE,
  ...
)

```

Arguments

gr	GRanges-class
bsgenome	BSgenome-class
spacer	string: spacer pattern in extended IUPAC alphabet
pam	string: pam pattern in extended IUPAC alphabet
complement	TRUE (default) or FALSE: also search in compl ranges?
ontargetmethod	'Doench2016', 'Doench2016' or NULL (no on-target score)

offtargetmethod
 'bowtie', 'pdict', or NULL (no offtarget analysis)
 offtargetfilterby
 filter for best off-target counts by this variable
 subtract_targets
 TRUE or FALSE (default): whether to subtract target (mis)matches from offtarget counts
 mismatches
 0-3: allowed mismatches in offtargetanalysis (choose mismatch=-1 to suppress offtarget analysis)
 indexedgenomesdir
 directory with Bowtie-indexed genomes (as produced with [index_genome](#))
 outdir
 directory where bowtie analysis results are written to
 verbose
 TRUE (default) or FALSE
 plot
 TRUE (default) or FALSE
 ...
 passed to plot_intervals

Value

[GRanges-class](#)

See Also

[find_primespacers](#) to find prime editing spacers

Examples

```

# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                       HBB  = 'chr11:5227002:-',          # snp
                       HEXA = 'chr15:72346580-72346583:-', # del
                       CFTR = 'chr7:117559593-117559595:+', # ins
                       bsgenome))

plot_intervals(gr)
find_primespacers(gr, bsgenome)
find_spacers(extend_for_pe(gr), bsgenome, complement=FALSE, mismatches=0)
# complement = FALSE because extend_for_pe already
# adds reverse complements and does so in a strand-specific
# manner

# TFBS example
#-----
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, 'mm10') %>% extend()
gr %<>% extract(1:100)
find_spacers(gr, bsgenome, subtract_targets = TRUE)

```

genes_to_granges	<i>Convert geneids into GRanges</i>
------------------	-------------------------------------

Description

Convert geneids into GRanges

Usage

```
genes_to_granges(geneids, txdb, complement = TRUE, plot = TRUE, verbose = TRUE)
```

```
genefile_to_granges(file, txdb, complement = TRUE, plot = TRUE)
```

Arguments

geneids	Gene identifier vector
txdb	TxDb-class or EnsDb-class
complement	TRUE (default) or FALSE: add complementary strand?
plot	TRUE (default) or FALSE
verbose	TRUE (default) or FALSE
file	Gene identifier file (one per row)

Value

[GRanges-class](#)

See Also

[char_to_granges](#), [bed_to_granges](#)

Examples

```
# Entrez
#-----
genefile <- system.file('extdata/SRF.entrez', package='multicrispr')
geneids  <- as.character(read.table(genefile)[[1]])
txdb     <- getFromNamespace('TxDb.Mmusculus.UCSC.mm10.knownGene',
                             'TxDb.Mmusculus.UCSC.mm10.knownGene')
(gr <- genes_to_granges(geneids, txdb))
(gr <- genefile_to_granges(genefile, txdb))

# Ensembl
#-----
# txdb <- AnnotationHub::AnnotationHub()[["AH75036"]]
# genefile <- system.file('extdata/SRF.ensembl', package='multicrispr')
# geneids <- as.character(read.table(genefile)[[1]])
# (gr <- genes_to_granges(geneids, txdb))
# (gr <- genefile_to_granges(genefile, txdb))
```

gr2dt	<i>GRanges</i> <-> <i>data.table</i>
-------	--------------------------------------

Description

GRanges <-> data.table

Usage

gr2dt(gr)

dt2gr(dt, seqinfo)

Arguments

gr [GRanges-class](#)
dt data.table
seqinfo [Seqinfo-class](#)

Value

data.table (gr2dt) or GRanges (dt2gr)

Examples

```
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                        HBB  = 'chr11:5227002:-',          # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+', # ins
                        bsgenome)
(dt <- gr2dt(gr))
(gr <- dt2gr(dt, BSgenome::seqinfo(bsgenome)))
```

has_been_indexed	<i>Has been indexed?</i>
------------------	--------------------------

Description

Has been indexed?

Usage

has_been_indexed(bsgenome, indexedgenomesdir = INDEXEDGENOMESDIR)

Arguments

bsgenome BSgenome
 indexedgenomesdir
 directory with indexed genomes

Value

TRUE or FALSE

Examples

```
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
has_been_indexed(bsgenome)
```

index_genome	<i>Index genome</i>
--------------	---------------------

Description

Bowtie index genome

Usage

```
index_genome(
  bsgenome,
  indexedgenomesdir = INDEXEDGENOMESDIR,
  download = TRUE,
  overwrite = FALSE
)
```

Arguments

bsgenome [BSgenome-class](#)
 indexedgenomesdir
 string: directory with bowtie-indexed genome
 download TRUE (default) or FALSE: whether to download pre-indexed version if available
 overwrite TRUE or FALSE (default)

Details

Checks whether already available locally. If not, checks whether indexed version can be downloaded from our s3 storage. If not, builds the index with bowtie. This can take a few hours, but is a one-time operation.

Value

invisible(genomdir)

Examples

```
bsgenome <- BSgenome.Scerevisiae.UCSC.sacCer1::Scerevisiae
index_genome(bsgenome, indexedgenomesdir = tempdir())
```

index_targets	<i>Index targets</i>
---------------	----------------------

Description

Bowtie index targets

Usage

```
index_targets(
  targets,
  bsgenome = getBSgenome(genome(targets)[1]),
  outdir = OUTDIR,
  verbose = TRUE
)
```

Arguments

targets	GRanges-class
bsgenome	BSgenome-class
outdir	string: output directory
verbose	TRUE (default) or FALSE

Value

invisible(targetdir)

Examples

```
require(magrittr)
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
targets <- extend(bed_to_granges(bedfile, genome = 'mm10'))
index_targets(targets, bsgenome)
```

plot_intervals	<i>Interval plot GRanges</i>
----------------	------------------------------

Description

Interval plot GRanges

Usage

```
plot_intervals(
  gr,
  xref = "targetname",
  y = default_y(gr),
  nperchrom = 2,
  nchrom = 4,
  color_var = "targetname",
  facet_var = "seqnames",
  linetype_var = default_linetype(gr),
  size_var = default_size_var(gr),
  alpha_var = default_alpha_var(gr),
  title = NULL,
  scales = "free"
)
```

Arguments

gr	GRanges-class
xref	gr var used for scaling x axis
y	'names' (default) or name of gr variable
nperchrom	number (default 1): n head (and n tail) targets shown per chromosome
nchrom	number (default 6) of chromosomes shown
color_var	'seqnames' (default) or other gr variable
facet_var	NULL(default) or gr variable mapped to facet
linetype_var	NULL (default) or gr variable mapped to linetype
size_var	NULL (default) or gr variable mapped to size
alpha_var	NULL or gr variable mapped to alpha
title	NULL or string: plot title
scales	'free', 'fixed', etc

Value

ggplot object

See Also

[plot_karyogram](#)

Examples

```
# SRF sites
require(magrittr)
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
targets <- bed_to_granges(bedfile, 'mm10', plot = FALSE)
plot_intervals(targets)

# PE targets
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',
                        HBB = 'chr11:5227002:-',
                        HEXA = 'chr15:72346580-72346583:-',
                        CFTR = 'chr7:117559593-117559595:+'),
                      bsgenome)
spacers <- find_primespacers(gr, bsgenome, plot = FALSE)
plot_intervals(gr)
plot_intervals(extend_for_pe(gr))
plot_intervals(spacers)

# Empty gr
plot_intervals(GenomicRanges::GRanges())
```

plot_karyogram	<i>Karyo/Interval Plot GRanges(List)</i>
----------------	--

Description

Karyo/Interval Plot GRanges(List)

Usage

```
plot_karyogram(grlist, title = unique(genome(grlist)))
```

Arguments

grlist	GRanges-class
title	plot title

Value

list

See Also[plot_intervals](#)**Examples**

```
# Plot GRanges
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
gr <- bed_to_granges(bedfile, 'mm10', plot = FALSE)
plot_karyogram(gr)

# Plot GRangesList
flanks <- up_flank(gr, stranded=FALSE)
grlist <- GenomicRanges::GRangesList(sites = gr, flanks = flanks)
plot_karyogram(grlist)
```

score_ontargets	<i>Add on-target efficiency scores</i>
-----------------	--

Description

Add Doench2014 or Doench2016 on-target efficiency scores

Usage

```
score_ontargets(
  spacers,
  bsgenome,
  ontargetmethod = c("Doench2014", "Doench2016")[1],
  chunksize = 10000,
  verbose = TRUE,
  plot = TRUE,
  ...
)
```

Arguments

spacers	GRanges-class : spacers
bsgenome	BSgenome-class
ontargetmethod	'Doench2014' (default) or 'Doench2016' (requires non-NULL argument python, virtualenv, or condaenv)
chunksize	Doench2016 is executed in chunks of chunksize
verbose	TRUE (default) or FALSE
plot	TRUE (default) or FALSE
...	passed to plot_intervals

Details

add_ontargets adds efficiency scores filter_ontargets adds efficiency scores and filters on them

Value

numeric vector

References

Doench 2014, Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. Nature Biotechnology, doi: 10.1038/nbt.3026

Doench 2016, Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nature Biotechnology, doi: 10.1038/nbt.3437

Python module azimuth: [github/MicrosoftResearch/azimuth](https://github.com/MicrosoftResearch/azimuth)

Examples

```
# Install azimuth
#-----
## With reticulate
# require(reticulate)
# conda_create('azienv', c('python=2.7'))
# use_condaenv('azienv')
# py_install(c('azimuth', 'scikit-learn==0.17.1', 'biopython==1.76'),
#           'azienv', pip = TRUE)

## Directly
# conda create --name azienv python=2.7
# conda activate azienv
# pip install scikit-learn==0.17.1
# pip install biopython==1.76
# pip install azimuth

# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
targets <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                             HBB  = 'chr11:5227002:-',        # snp
                             HEXA = 'chr15:72346580-72346583:-', # del
                             CFTR = 'chr7:117559593-117559595:+', # ins
                             bsgenome)
spacers <- find_primespacers(targets, bsgenome, ontargetmethod=NULL,
                             offtargetmethod=NULL)
spacers %>% score_ontargets(bsgenome, 'Doench2014')
# reticulate::use_condaenv('azienv')
# reticulate::import('azimuth')
# spacers %>% score_ontargets(bsgenome, 'Doench2016')

# TFBS example
```

```
#-----
bedfile <- system.file('extdata/SRF.bed', package = 'multicrispr')
bsgenome <- BSgenome.Mmusculus.UCSC.mm10::BSgenome.Mmusculus.UCSC.mm10
targets <- extend(bed_to_granges(bedfile, 'mm10'))
spacers <- find_spacers(targets, bsgenome, ontargetmethod=NULL,
                        offtargetmethod=NULL)

spacers %<>% score_ontargets(bsgenome, 'Doench2014')
# reticulate::use_condaenv('azienv')
# reticulate::import('azimuth')
# spacers %>% score_ontargets(bsgenome, 'Doench2016')
```

up_flank

Extend or Flank GRanges

Description

Returns extensions, upstream flanks, or downstream flanks

Usage

```
up_flank(
  gr,
  start = -200,
  end = -1,
  strandaware = TRUE,
  bsgenome = NULL,
  verbose = FALSE,
  plot = FALSE,
  linetype_var = "set",
  ...
)

down_flank(
  gr,
  start = 1,
  end = 200,
  strandaware = TRUE,
  bsgenome = NULL,
  verbose = FALSE,
  plot = FALSE,
  linetype_var = "set",
  ...
)

extend(
  gr,
  start = -22,
  end = 22,
```

```

    strandaware = TRUE,
    bsgenome = NULL,
    verbose = FALSE,
    plot = FALSE,
    linetype_var = "set",
    ...
)

```

Arguments

gr	GRanges-class
start	number or vector (same length as gr): start definition, relative to gr start (up_flank, extend) or gr end (down_flank).
end	number or vector (same length as gr): end definition, relative to gr start (up_flank) or gr end (extend, down_flank).
strandaware	TRUE (default) or FALSE: consider strand information?
bsgenome	NULL (default) or BSgenome-class . Required to update gr\$seq if present.
verbose	TRUE or FALSE (default)
plot	TRUE or FALSE (default)
linetype_var	string: gr var mapped to linetype
...	passed to plot_intervals

Details

up_flank returns upstream flanks, in relation to start(gr). down_flank returns downstream flanks, in relation to end(gr). extend returns extensions, in relation to start(gr) and end(gr)

Value

a [GRanges-class](#)

Examples

```

# PE example
#-----
require(magrittr)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(PRNP = 'chr20:4699600:+',          # snp
                        HBB  = 'chr11:5227002:-',          # snp
                        HEXA = 'chr15:72346580-72346583:-', # del
                        CFTR = 'chr7:117559593-117559595:+'), # ins
                      bsgenome = bsgenome)

gr %>% up_flank( -22, -1, plot=TRUE)
gr %>% up_flank( c(-10,-20,-30,-40), -1, plot=TRUE)
gr %>% up_flank( -22, -1, plot=TRUE, strandaware=FALSE)

gr %>% down_flank(+1, +22, plot=TRUE)
gr %>% down_flank(+1, c(10, 20, 30, 40), plot=TRUE)

```

```

gr %>% down_flank(+1, +22, plot=TRUE, strandaware=FALSE)

gr %>% extend( -10, +20, plot=TRUE)
gr %>% extend( -10, +20, plot=TRUE, strandaware=FALSE)

# TFBS example
#-----
bedfile <- system.file('extdata/SRF.bed', package='multicrispr')
gr <- bed_to_granges(bedfile, genome = 'mm10')
gr %>% extend(plot = TRUE)
gr %>% up_flank(plot = TRUE)
gr %>% down_flank(plot = TRUE)

```

write_ranges	<i>Write GRanges to file</i>
--------------	------------------------------

Description

Write GRanges to file

Usage

```
write_ranges(gr, file, verbose = TRUE)
```

```
read_ranges(file, bsgenome)
```

Arguments

gr	GRanges-class
file	file
verbose	TRUE (default) or FALSE
bsgenome	BSgenome-class

Value

[GRanges-class](#) for read_ranges

Examples

```

# Find PE spacers for 4 clinically relevant loci (Anzalone et al, 2019)
bsgenome <- BSgenome.Hsapiens.UCSC.hg38::BSgenome.Hsapiens.UCSC.hg38
gr <- char_to_granges(c(
  PRNP = 'chr20:4699600:+',          # snp: prion disease
  HBB  = 'chr11:5227002:-',          # snp: sickle cell anemia
  HEXA = 'chr15:72346580-72346583:-', # del: tay sachs disease
  CFTR = 'chr7:117559593-117559595:+'), # ins: cystic fibrosis
  bsgenome)
file <- file.path(tempdir(), 'gr.txt')
write_ranges(gr, file)
read_ranges(file, bsgenome)

```

Index

`add_genome_matches`, [2](#)
`add_inverse_strand`, [4](#)
`add_seq`, [5](#)
`add_target_matches`, [6](#)

`bed_to_granges`, [7](#), [8](#), [18](#)
`BSgenome`, [12](#)

`char_to_granges`, [7](#), [8](#), [18](#)

`double_flank`, [8](#)
`down_flank (up_flank)`, [26](#)
`dt2gr (gr2dt)`, [19](#)

`extend (up_flank)`, [26](#)
`extend_for_pe`, [10](#)
`extend_pe_to_gg`, [11](#)
`extract_matchranges`, [12](#)
`extract_subranges`, [13](#)

`find_gg`, [13](#)
`find_primespacers`, [14](#), [17](#)
`find_spacers`, [15](#), [16](#)

`genefile_to_granges (genes_to_granges)`,
 [18](#)
`genes_to_granges`, [7](#), [8](#), [18](#)
`gr2dt`, [19](#)

`has_been_indexed`, [19](#)

`index_genome`, [15](#), [17](#), [20](#)
`index_targets`, [21](#)

`plot_intervals`, [4](#), [22](#), [24](#), [27](#)
`plot_karyogram`, [23](#), [23](#)

`read_ranges (write_ranges)`, [28](#)

`score_ontargets`, [24](#)

`up_flank`, [26](#)

`write_ranges`, [28](#)