Package 'CRISPRseek'

October 3, 2021

Type Package

Title Design of target-specific guide RNAs in CRISPR-Cas9,
genome-editing systems

Version 1.32.0

Date 2021-04-09

Encoding UTF-8

Author
Lihua Julie Zhu, Benjamin R. Holmes, Hervé Pagès, Hui Mao, Michael Lawrence, Isana Veksler-Lublinsky, Victor Ambros, Neil Aronin and Michael Brodsky

Maintainer Lihua Julie Zhu <julie.zhu@umassmed.edu>

Depends R (>= 3.0.1), BiocGenerics, Biostrings

Imports parallel, data.table, seqinr, S4Vectors (>= 0.9.25), IRanges,
BSgenome, BiocParallel, hash, methods,reticulate,rhdf5

Suggests RUnit, BiocStyle, BSgenome.Hsapiens.UCSC.hg19,
TxDb.Hsapiens.UCSC.hg19.knownGene, org.Hs.eg.db

Description The package includes functions to find potential guide RNAs for the CRISPR editing system including Base Editors and the Prime Editor for input target sequences, optionally filter guide RNAs without restriction enzyme cut site, or without paired guide RNAs, genome-wide search for off-targets, score, rank, fetch flank sequence and indicate whether the target and off-targets are located in exon region or not. Potential guide RNAs are annotated with total score of the top5 and topN off-targets, detailed topN mismatch sites, restriction enzyme cut sites, and paired guide RNAs. The package also output indels and their frequencies for Cas9 targeted sites.

License GPL (>= 2)

LazyLoad yes

biocViews ImmunoOncology, GeneRegulation, SequenceMatching, CRISPR

RoxygenNote 7.1.0

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

git_branch RELEASE_3_13

git_last_commit b64de86

git_last_commit_date 2021-05-19

Date/Publication 2021-10-03

R topics documented:

CRISPRseek–package   *Design of target-specific guide RNAs (gRNAs) in CRISPR-Cas9,*
                             genome-editing systems

Description

Design of target-specific gRNAs for the CRISPR-Cas9 system by automatically finding potential
gRNAs (paired/not paired), with/without restriction enzyme cut site(s) in a given sequence, search-
ing for off targets with user defined maximum number of mismatches, calculating score of each
off target based on mismatch positions in the off target and a penalty weight matrix, filtering off
targets with user-defined criteria, and annotating off targets with flank sequences, whether located
in exon or not. Summary report is also generated with gRNAs ranked by total topN off target score,
annotated with restriction enzyme cut sites, gRNA efficacy and possible paired gRNAs. Detailed
paired gRNAs information and restriction enzyme cut sites are stored in separate files in the output
directory specified by the user. In total, four tab delimited files are generated in the output direc-
tory: OfftargetAnalysis.xls (off target details), Summary.xls (gRNA summary), REcutDetails.xls
(restriction enzyme cut sites of each gRNA), and pairedgRNAs.xls (potential paired gRNAs).

Details

Package:	CRISPRseek
Type:	Package
Version:	1.0
Date:	2013-10-04

License: GPL (>= 2)

Function offTargetAnalysis integrates all steps of off target analysis into one function call

Author(s)

Lihua Julie Zhu and Michael Brodsky Maintainer: julie.zhu@umassmed.edu

References

Mali P, Aach J, Stranges PB, Esvelt KM, Moosburner M, Kosuri S, Yang L, Church GM.CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat Biotechnol. 2013. 31(9):833-8 Patrick D Hsu, David A Scott, Joshua A Weinstein, F Ann Ran, Silvana Konermann, Vineeta Agarwala, Yinqing Li, Eli J Fine, Xuebing Wu, Ophir Shalem, Thomas J Cradick, Luciano A Marraffini, Gang Bao & Feng Zhang. DNA targeting specificity of rNA-guided Cas9 nucleases. Nat Biotechnol. 2013. 31:827-834 Lihua Julie Zhu, Benjamin R. Holmes, Neil Aronin and Michael Brodsky. CRISPRseek: a Bioconductor package to identify target-specific guide RNAs for CRISPR-Cas9 genome-editing systems. Plos One Sept 23rd 2014 Doench JG et al., Optimized sgRNA design to maximize activity and minimize off-target effe cts of CRISPR-Cas9. Nature Biotechnology Jan 18th 2016

See Also

offTargetAnalysis

Examples

```
library(CRISPRseek)
library("BSgenome.Hsapiens.UCSC.hg19")
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
library(org.Hs.eg.db)
outputDir <- getwd()
inputFilePath <- system.file("extdata", "inputseq.fa", package = "CRISPRseek")
REpatternFile <- system.file("extdata", "NEBenzymes.fa", package = "CRISPRseek")
######## Scenario 1. Target and off-target analysis for paired gRNAs with
######## one of the pairs overlap RE sites
results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly=TRUE,
    REpatternFile =REpatternFile,findPairedgRNAOnly=TRUE,
    BSgenomeName=Hsapiens, txdb=TxDb.Hsapiens.UCSC.hg19.knownGene,
    orgAnn = org.Hs.egSYMBOL,max.mismatch = 1, chromToSearch = "chrX",
    outputDir = outputDir,overwrite = TRUE)

######## Scenario 2. Target and off-target analysis for paired gRNAs with or
######## without RE sites
results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly = FALSE,
    REpatternFile = REpatternFile,findPairedgRNAOnly = TRUE,
    BSgenomeName = Hsapiens, txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
    orgAnn = org.Hs.egSYMBOL,max.mismatch = 1, chromToSearch = "chrX",
    outputDir = outputDir, overwrite = TRUE)
```

```
######## Scenario 3. Target and off-target analysis for gRNAs overlap RE sites

    results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly = TRUE,
        REpatternFile = REpatternFile,findPairedgRNAOnly = FALSE,
        BSgenomeName = Hsapiens, txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
        orgAnn = org.Hs.egSYMBOL, max.mismatch = 1, chromToSearch = "chrX",
        outputDir = outputDir, overwrite = TRUE)

######## Scenario 4. Off-target analysis for all potential gRNAs, this will
########be the slowest among the aforementioned scenarios.

    results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly = FALSE,
        REpatternFile = REpatternFile,findPairedgRNAOnly = FALSE,
        BSgenomeName = Hsapiens, txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
         orgAnn = org.Hs.egSYMBOL, max.mismatch = 1, chromToSearch = "chrX",
        outputDir = outputDir,overwrite = TRUE)

######## Scenario 5. Target and off-target analysis for gRNAs input by user.
    gRNAFilePath <- system.file("extdata", "testHsap_GATA1_ex2_gRNA1.fa",
        package="CRISPRseek")
    results <- offTargetAnalysis(inputFilePath = gRNAFilePath, findgRNAs = FALSE,
        findgRNAsWithREcutOnly = FALSE, REpatternFile = REpatternFile,
        findPairedgRNAOnly = FALSE, BSgenomeName = Hsapiens,
        txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
         orgAnn = org.Hs.egSYMBOL, max.mismatch = 1, chromToSearch = "chrX",
        outputDir = outputDir, overwrite = TRUE)

####### Scenario 6. Quick gRNA finding  without target and off-target analysis
    results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly = TRUE,
        REpatternFile = REpatternFile,findPairedgRNAOnly = TRUE,
        chromToSearch = "", outputDir = outputDir, overwrite = TRUE)

####### Scenario 7. Quick gRNA finding  with gRNA efficacy analysis
    results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly = TRUE,
        REpatternFile = REpatternFile,findPairedgRNAOnly = TRUE,
BSgenomeName = Hsapiens, annotateExon = FALSE,
        max.mismatch = 0, outputDir = outputDir, overwrite = TRUE)
```

---

annotateOffTargets   *annotate off targets*

Description

annotate off targets to indicate whether it is inside an exon or intron, and the gene id if inside the gene.

Usage

```
annotateOffTargets(scores, txdb, orgAnn, ignore.strand = TRUE)
```

Arguments

scores	a data frame output from getOfftargetScore or filterOfftarget. It contains strand (strand of the off target, + for plus and - for minus strand), chrom (chromosome of the off target), chromStart (start position of the off target), chromEnd (end position of the off target),name (gRNA name),gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), OffTargetSequence (the genomic sequence of the off target), n.mismatch (number of mismatches between the off target and the gRNA), forViewInUCSC (string for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score (score of the off target), mismatch.distance2PAM (a comma separated distances of all mismatches to PAM, e.g., 14,11 means one mismatch is 14 bp away from PAM and the other mismatch is 11 bp away from PAM), alignment (alignment between gRNA and off target, e.g., ...G..C.......... means that this off target aligns with gRNA except that G and C are mismatches),NGG (this off target contains canonical PAM or not, 1 for yes and 0 for no) mean.neighbor.distance.mismatch (mean distance between neighboring mismatches)
txdb	TxDb object, for creating and using TxDb object, please refer to GenomicFeatures package. For a list of existing TxDb object, please search for annotation package starting with Txdb at http://www.bioconductor.org/packages/release/BiocViews.html#___Annota such as TxDb.Rnorvegicus.UCSC.rn5.refGene for rat, TxDb.Mmusculus.UCSC.mm10.knownGene for mouse, TxDb.Hsapiens.UCSC.hg19.knownGene for human, TxDb.Dmelanogaster.UCSC.dm3.ensGer for Drosophila and TxDb.Celegans.UCSC.ce6.ensGene for C.elegans
orgAnn	organism annotation mapping such as org.Hs.egSYMBOL in org.Hs.eg.db package for human
ignore.strand	default to TRUE

Value

a data frame with off target annotation

Author(s)

Lihua Julie Zhu

References

Lihua Julie Zhu, Benjamin R. Holmes, Neil Aronin and Michael Brodsky. CRISPRseek: a Bioconductor package to identify target-specific guide RNAs for CRISPR-Cas9 genome-editing systems. Plos One Sept 23rd 2014

See Also

offTargetAnalysis

Examples

```
library(CRISPRseek)
#library("BSgenome.Hsapiens.UCSC.hg19")
```

```
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
library(org.Hs.eg.db)
hitsFile <-  system.file("extdata", "hits.txt", package="CRISPRseek")
hits <- read.table(hitsFile, sep = "\t", header = TRUE,
    stringsAsFactors = FALSE)
featureVectors <- buildFeatureVectorForScoring(hits)
scores <- getOfftargetScore(featureVectors)
outputDir <- getwd()
results <- annotateOffTargets(scores,
    txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
     orgAnn = org.Hs.egSYMBOL)
results
```

buildFeatureVectorForScoring

Build feature vectors

Description

Build feature vectors for calculating scores of off targets

Usage

```
buildFeatureVectorForScoring(hits, gRNA.size = 20,
    canonical.PAM = "NGG",
    subPAM.position = c(22,23), PAM.size = 3, PAM.location = "3prime")
```

Arguments

hits	a data frame generated from searchHits, which contains IsMismatch.posX (Indicator variable indicating whether this position X is mismatch or not, 1 means yes and 0 means not, X = 1- gRNA.size) representing all positions in the guide RNA, abbreviated as gRNA),strand (strand of the off target, + for plus and - for minus strand), chrom (chromosome of the off target), chromStart (start position of the off target), chromEnd (end position of the off target),name (gRNA name), gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), OffTargetSequence (the genomic sequence of the off target), n.mismatch (number of mismatches between the off target and the gRNA), forViewInUCSC (string for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score (set to 100, and will be calculated in getOfftargetScore)
gRNA.size	gRNA size, default 20
canonical.PAM	Canonical PAM, default NGG for spCas9, TTTN for Cpf1
subPAM.position	
	The start and end positions of the sub PAM to fetch. Default to 22 and 23 for SP with 20bp gRNA and NGG as preferred PAM
PAM.size	Size of PAM, default to 3 for spCas9, 4 for Cpf1
PAM.location	PAM location relative to gRNA. For example, default to 3prime for spCas9 PAM. Please set to 5prime for cpf1 PAM since it's PAM is located on the 5 prime end

Value

A data frame with hits plus features used for calculating scores and for generating report, including IsMismatch.posX (Indicator variable indicating whether this position X is mismatch or not, 1 means yes and 0 means not, X = 1- gRNA.size) representing all positions in the gRNA), strand (strand of the off target, + for plus and - for minus strand),chrom (chromosome of the off target), chromStart (start position of the off target),chromEnd (end position of the off target),name (gRNA name), gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), OffTarget-Sequence (the genomic sequence of the off target), n.mismatch (number of mismatches between the off target and the gRNA), forViewInUCSC (string for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score (score of the off target), mismatche.distance2PAM (a comma separated distances of all mismatches to PAM, e.g., 14,11 means one mismatch is 14 bp away from PAM and the other mismatch is 11 bp away from PAM), alignment (alignment between gRNA and off target, e.g., ...G..C.......... means that this off target aligns with gRNA except that G and C are mismatches), NGG (this off target contains canonical PAM or not, 1 for yes and 0 for no) mean.neighbor.distance.mismatch (mean distance between neighboring mismatches)

Author(s)

Lihua Julie Zhu

See Also

offTargetAnalysis

Examples

```
hitsFile <-  system.file("extdata", "hits.txt", package = "CRISPRseek")
hits <- read.table(hitsFile, sep= "\t", header = TRUE,
    stringsAsFactors = FALSE)
buildFeatureVectorForScoring(hits)
```

---

calculategRNAEfficiency

Calculate gRNA Efficiency

Description

Calculate gRNA Efficiency for a given set of sequences and feature weight matrix

Usage

```
calculategRNAEfficiency(extendedSequence,
    baseBeforegRNA, featureWeightMatrix, gRNA.size = 20,
    enable.multicore = FALSE, n.cores.max = 6)
```

Arguments

extendedSequence

> Sequences containing gRNA plus PAM plus flanking sequences. Each sequence should be long enough for building features specified in the featureWeightMatrix

baseBeforegRNA   Number of bases before gRNA used for calculating gRNA efficiency, default 4

featureWeightMatrix

> a data frame with the first column containing significant features and the second column containing the weight of corresponding features. In the following example, DoenchNBT2014 weight matrix is used. Briefly, features include INTERCEPT,GC_LOW (penalty for low GC content in the gRNA sequence), GC_HIGH (penalty for high GC content in the gRNA sequence), G02 (means G at second position of the extendedSequence), GT02 (means GT di-nucleotides starting at 2nd position of the extendedSequence). To understand how is the feature weight matrix is identified, or how to use alternative feature weight matrix file, please see Doench et al., 2014 for details.

gRNA.size   The size of the gRNA, default 20

enable.multicore

> Indicate whether enable parallel processing, default FALSE. For super long sequences with lots of gRNAs, suggest set it to TRUE

n.cores.max   Indicating maximum number of cores to use in multi core mode, i.e., parallel processing, default 6. Please set it to 1 to disable multicore processing for small dataset.

Value

DNAStringSet consists of potential gRNAs that can be input to filtergRNAs function directly

Author(s)

Lihua Julie Zhu

References

Doench JG, Hartenian E, Graham DB, Tothova Z, Hegde M, Smith I, Sullender M, Ebert BL, Xavier RJ, Root DE. Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. Nat Biotechnol. 2014 Sep 3. doi: 10.1038 nbt.3026 http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design

See Also

offTargetAnalysis

Examples

```
  extendedSequence <- c("TGGATTGTATAATCAGCATGGATTTGGAAC",
"TCAACGAGGATATTCTCAGGCTTCAGGTCC",
"GTTACCTGAATTTGACCTGCTCGGAGGTAA",
"CTTGGTGTGGCTTCCTTTAAGACATGGAGC",
```

```
"CATACAGGCATTGAAGAAGAATTTAGGCCT",
"AGTACTATACATTTGGCTTAGATTTGGCGG",
"TTTTCCAGATAGCCGATCTTGGTGTGGCTT",
"AAGAAGGGAACTATTCGCTGGTGATGGAGT"
)
featureWeightMatrixFile <- system.file("extdata", "DoenchNBT2014.csv",
package = "CRISPRseek")
featureWeightMatrix <- read.csv(featureWeightMatrixFile, header=TRUE)
calculategRNAEfficiency(extendedSequence, baseBeforegRNA = 4,
featureWeightMatrix, gRNA.size = 20)
```

compare2Sequences	*Compare 2 input sequences/sequence sets for possible guide RNAs (gRNAs)*

Description

Generate all possible guide RNAs (gRNAs) for two input sequences, or two sets of sequences and generate scores for potential off-targets in the other sequence.

Usage

```
compare2Sequences(inputFile1Path, inputFile2Path,
    inputNames=c("Seq1", "Seq2"),
    format = c("fasta", "fasta"), header=FALSE, findgRNAsWithREcutOnly = FALSE,
    searchDirection=c("both","1to2", "2to1"), BSgenomeName,
    baseEditing = FALSE, targetBase = "C", editingWindow = 4:8,
    editingWindow.offtargets = 4:8,
  REpatternFile=system.file("extdata", "NEBenzymes.fa", package = "CRISPRseek"),
  minREpatternSize = 6, findgRNAs = c(TRUE, TRUE), removegRNADetails = c(FALSE, FALSE),
   exportAllgRNAs = c("no", "all", "fasta", "genbank"), annotatePaired =  FALSE,
   overlap.gRNA.positions = c(17, 18), findPairedgRNAOnly = FALSE,
   min.gap = 0, max.gap = 20, gRNA.name.prefix = "_gR", PAM.size = 3,
   gRNA.size = 20, PAM = "NGG", PAM.pattern = "NNG$|NGN$",
   allowed.mismatch.PAM = 1, max.mismatch = 3,
   outputDir, upstream = 0, downstream = 0,
   weights = c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445,
   0.508, 0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583),
   overwrite = FALSE, baseBeforegRNA = 4,
   baseAfterPAM = 3, featureWeightMatrixFile = system.file("extdata",
       "DoenchNBT2014.csv", package = "CRISPRseek"), foldgRNAs = FALSE,
   gRNA.backbone =
"GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU",
   temperature = 37,
   scoring.method = c("Hsu-Zhang", "CFDscore"),
       subPAM.activity = hash( AA =0,
```

```
             AC =   0,
             AG = 0.259259259,
             AT = 0,
             CA = 0,
             CC = 0,
             CG = 0.107142857,
             CT = 0,
             GA = 0.069444444,
             GC = 0.022222222,
             GG = 1,
             GT = 0.016129032,
             TA = 0,
             TC = 0,
             TG = 0.038961039,
             TT = 0),
        subPAM.position = c(22, 23),
      PAM.location = "3prime",
    rule.set = c("Root_RuleSet1_2014", "Root_RuleSet2_2016"), mismatch.activity.file = system.file("ex
          "NatureBiot2016SuppTable19DoenchRoot.csv",
          package = "CRISPRseek")
    )
```

Arguments

inputFile1Path	Sequence input file 1 path that contains one of the two sequences to be searched for potential gRNAs. It can also be a DNAStringSet object with names field set. Please see examples below.
inputFile2Path	Sequence input file 2 path that contains one of the two sequences to be searched for potential gRNAs. It can also be a DNAStringSet object with names field set. Please see examples below.
inputNames	Name of the input sequences when inputFile1Path and inputFile2Path are DNAStringSet instead of file path
format	Format of the input files, fasta, fastq and bed format are supported, default fasta
header	Indicate whether the input file contains header, default FALSE, only applies to bed format
findgRNAsWithREcutOnly	
	Indicate whether to find gRNAs overlap with restriction enzyme recognition pattern
searchDirection	
	Indicate whether perfrom gRNA in both sequences and off-target search against each other (both) or search gRNA in input1 and off-target analysis in input2 (1to2), or vice versa (2to1)
BSgenomeName	BSgenome object. Please refer to available.genomes in BSgenome package. For example, BSgenome.Hsapiens.UCSC.hg19 for hg19, BSgenome.Mmusculus.UCSC.mm10 for mm10, BSgenome.Celegans.UCSC.ce6 for ce6, BSgenome.Rnorvegicus.UCSC.rn5 for rn5, BSgenome.Drerio.UCSC.danRer7 for Zv9, and BSgenome.Dmelanogaster.UCSC.dm3 for dm3

baseEditing	Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE, please set baseEditing = TRUE, targetBase and editingWidow accordingly.
targetBase	Applicable only when baseEditing is set to TRUE. It is used to indicate the target base for base editing systems, default to C for converting C to T in the CBE system. Please change it to A if you intend to use the ABE system.
editingWindow	Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window.
editingWindow.offtargets	
	Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window to consider for the offtargets search only, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window, or you would like to include offtargets with the target base in a larger editing window.
REpatternFile	File path containing restriction enzyme cut patters
minREpatternSize	
	Minimum restriction enzyme recognition pattern length required for the enzyme pattern to be searched for, default 6
findgRNAs	Indicate whether to find gRNAs from the sequences in the input file or skip the step of finding gRNAs, default TRUE for both input sequences. Set it to FALSE if the input file contains user selected gRNAs plus PAM already.
removegRNADetails	
	Indicate whether to remove the detailed gRNA information such as efficacy file and restriction enzyme cut sites, default false for both input sequences. Set it to TRUE if the input file contains the user selected gRNAs plus PAM already.
exportAllgRNAs	Indicate whether to output all potential gRNAs to a file in fasta format, genbank format or both. Default to no.
annotatePaired	Indicate whether to output paired information, default to FALSE
overlap.gRNA.positions	
	The required overlap positions of gRNA and restriction enzyme cut site, default 17 and 18
findPairedgRNAOnly	
	Choose whether to only search for paired gRNAs in such an orientation that the first one is on minus strand called reverse gRNA and the second one is on plus strand called forward gRNA. TRUE or FALSE, default FALSE
min.gap	Minimum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 0
max.gap	Maximum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 20
gRNA.name.prefix	
	The prefix used when assign name to found gRNAs, default _gR, short for guided RNA.
PAM.size	PAM length, default 3
gRNA.size	The size of the gRNA, default 20

PAM	PAM sequence after the gRNA, default NGG
PAM.pattern	Regular expression of PAM, default NNG or NGN for spCas9. For cpf1, ^TTTN since it is a 5 prime PAM sequence
allowed.mismatch.PAM	
	Maximum number of mismatches allowed to the PAM sequence, default to 1 for PAM.pattern NNG or NGN PAM
max.mismatch	Maximum mismatch allowed to search the off targets in the other sequence, default 3
outputDir	the directory where the sequence comparison results will be written to
upstream	upstream offset from the bed input starts to search for gRNA and/or offtargets, default 0
downstream	downstream offset from the bed input ends to search for gRNA and/or offtargets, default 0
weights	numeric vector size of gRNA length, default c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445, 0.508, 0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583) which is used in Hsu et al., 2013 cited in the reference section
overwrite	overwrite the existing files in the output directory or not, default TRUE
baseBeforegRNA	Number of bases before gRNA used for calculating gRNA efficiency, default 4 Please note, for PAM located on the 5 prime, need to specify the number of bases before the PAM sequence plus PAM size.
baseAfterPAM	Number of bases after PAM used for calculating gRNA efficiency, default 3 for spCas9 Please note, for PAM located on the 5 prime, need to include the length of the gRNA plus the extended sequence on the 3 prime
featureWeightMatrixFile	
	Feature weight matrix file used for calculating gRNA efficiency. By default DoenchNBT2014 weight matrix is used. To use alternative weight matrix file, please input a csv file with first column containing significant features and the second column containing the corresponding weights for the features. Please see Doench et al., 2014 for details.
foldgRNAs	Default FALSE. If set to TRUE, summary file will contain minimum free energy of the secondary structure of gRNA with gRNA backbone from GeneRfold package provided that GeneRfold package has been installed.
gRNA.backbone	gRNA backbone constant region sequence. Default to the sequence in Sp gRNA backbone.
temperature	temperature in celsius. Default to 37 celsius.
scoring.method	Indicates which method to use for offtarget cleavage rate estimation, currently two methods are supported, Hsu-Zhang and CFDscore
subPAM.activity	
	Applicable only when scoring.method is set to CFDscore A hash to represent the cleavage rate for each alternative sub PAM sequence relative to preferred PAM sequence
subPAM.position	
	Applicable only when scoring.method is set to CFDscore The start and end positions of the sub PAM. Default to 22 and 23 for SP with 20bp gRNA and NGG as preferred PAM

PAM.location   PAM location relative to gRNA. For example, spCas9 PAM is located on the 3 prime (3prime) while cpf1 PAM is located on the 5 prime (5prime)

rule.set   Specify a rule set scoring system for calculating gRNA efficacy. Please note that Root_RuleSet2_2016 requires the following python packages with specified verion and python 2.7. 1. scikit-learn 0.16.1 2. pickle 3. pandas 4. numpy 5. scipy

mismatch.activity.file

Applicable only when scoring.method is set to CFDscore A comma separated (csv) file containing the cleavage rates for all possible types of single nucleotide mismatche at each position of the gRNA. By default, using the supplemental Table 19 from Doench et al., Nature Biotechnology 2016

Value

Return a data frame with all potential gRNAs from both sequences. In addition, a tab delimited file scoresFor2InputSequences.xls is also saved in the outputDir, sorted by scoreDiff descending.

name   name of the gRNA

gRNAPlusPAM   gRNA plus PAM sequence

targetInSeq1   target/off-target sequence including PAM in the 1st input sequence file

targetInSeq2   target/off-target sequence incuding PAM in the 2nd input sequence file

guideAlignment2Offtarget

alignment of gRNA to the other input sequence (off-target sequence)

offTargetStrand

strand of the other sequence (off-target sequence) the gRNA align to

scoreForSeq1   score for the target sequence in the 1st input sequence file

scoreForSeq2   score for the target sequence in the 1st input sequence file

mismatch.distance2PAM

distances of mismatch to PAM, e.g., 14 means the mismatch is 14 bp away from PAM

n.mismatch   number of mismatches between the off-target and the gRNA

targetSeqName   the name of the input sequence where the target sequence is located

scoreDiff   scoreForSeq1 - scoreForSeq2

bracket.notation

folded gRNA in bracket notation

mfe.sgRNA   minimum free energy of sgRNA

mfe.diff   mfe.sgRNA-mfe.backbone

mfe.backbone   minimum free energy of the gRNA backbone by itself

Author(s)

Lihua Julie Zhu

References

Patrick D Hsu, David A Scott, Joshua A Weinstein, F Ann Ran, Silvana Konermann, Vineeta Agar-
wala, Yinqing Li, Eli J Fine, Xuebing Wu, Ophir Shalem, Thomas J Cradick, Luciano A Marraffini,
Gang Bao & Feng Zhang (2013) DNA targeting specificity of rNA-guided Cas9 nucleases. Nature
Biotechnology 31:827-834

See Also

CRISPRseek

Examples

```
library(CRISPRseek)
inputFile1Path <- system.file("extdata", "rs362331T.fa",
        package = "CRISPRseek")
inputFile2Path <- system.file("extdata", "rs362331C.fa",
        package = "CRISPRseek")
REpatternFile <- system.file("extdata", "NEBenzymes.fa",
        package = "CRISPRseek")
seqs <- compare2Sequences(inputFile1Path, inputFile2Path,
    outputDir = getwd(),
    REpatternFile = REpatternFile, overwrite = TRUE)


seqs2 <- compare2Sequences(inputFile1Path, inputFile2Path,
        inputNames=c("Seq1", "Seq2"),
        scoring.method = "CFDscore",
        outputDir = getwd(),
        overwrite = TRUE, baseEditing = TRUE)


inputFile1Path <-
DNAStringSet(
"TAATATTTTAAAATCGGTGACGTGGGCCCAAAACGAGTGCAGTTCCAAAGGCACCCACCTGTGGCAG"
)
    ## when set inputFile1Path to a DNAStringSet object, it is important
    ## to call names
    names(inputFile1Path) <- "seq1"


    inputFile2Path <-
DNAStringSet(
"TAATATTTTAAAATCGGTGACGTGGGCCCAAAACGAGTGCAGTTCCAAAGGCACCCACCTGTGGCAG"
)
     ## when set inputFile2Path to a DNAStringSet object, it is important
    ## to call names

    names(inputFile2Path) <- "seq2"

    seqs <- compare2Sequences(inputFile1Path, inputFile2Path,
        inputNames=c("Seq1", "Seq2"),
        scoring.method = "CFDscore",
        outputDir = getwd(),
        overwrite = TRUE)
```

```
seqs2 <- compare2Sequences(inputFile1Path, inputFile2Path,
         inputNames=c("Seq1", "Seq2"),
         scoring.method = "CFDscore",
         outputDir = getwd(),
         overwrite = TRUE, baseEditing = TRUE)
```

---

filtergRNAs   *Filter gRNAs*

Description

Filter gRNAs containing restriction enzyme cut site

Usage

```
filtergRNAs(all.gRNAs, pairOutputFile = "",
    findgRNAsWithREcutOnly = FALSE,
    REpatternFile = system.file("extdata", "NEBenzymes.fa",
        package = "CRISPRseek"), format = "fasta",
    minREpatternSize = 4, overlap.gRNA.positions = c(17, 18),overlap.allpos = TRUE)
```

Arguments

all.gRNAs   gRNAs as DNAStringSet, such as the output from findgRNAs

pairOutputFile   File path with paired gRNAs

findgRNAsWithREcutOnly

        Indicate whether to find gRNAs overlap with restriction enzyme recognition pattern

REpatternFile   File path containing restriction enzyme cut patters

format   Format of the REpatternFile, default as fasta

minREpatternSize

        Minimum restriction enzyme recognition pattern length required for the enzyme pattern to be searched for, default 4

overlap.gRNA.positions

        The required overlap positions of gRNA and restriction enzyme cut site, default 17 and 18

overlap.allpos   Default TRUE, meaning that only gRNAs overlap with all the positions are retained FALSE, meaning that gRNAs overlap with one or both of the positions are retained

Value

gRNAs.withRE   gRNAs as DNAStringSet that passed the filter criteria

gRNAREcutDetails

        a data frame that contains a set of gRNAs annotated with restriction enzyme cut details

Author(s)

Lihua Julie Zhu

See Also

offTargetAnalysis

Examples

```
all.gRNAs <- findgRNAs(
    inputFilePath = system.file("extdata", "inputseq.fa",
    package = "CRISPRseek"),
    pairOutputFile = "testpairedgRNAs.xls",
    findPairedgRNAOnly = TRUE)

gRNAs.RE <- filtergRNAs(all.gRNAs = all.gRNAs,
    pairOutputFile = "testpairedgRNAs.xls",minREpatternSize = 6,
    REpatternFile = system.file("extdata", "NEBenzymes.fa",
    package = "CRISPRseek"), overlap.allpos = TRUE)

gRNAs   <- gRNAs.RE$gRNAs.withRE
restriction.enzyme.cut.sites <- gRNAs.RE$gRNAREcutDetails
```

---

filterOffTarget   *filter off targets and generate reports.*

Description

filter off targets that meet the criteria set by users such as minimum score, topN. In addition, off target was annotated with flank sequence, gRNA cleavage efficiency and whether it is inside an exon or not if fetchSequence is set to TRUE and annotateExon is set to TRUE

Usage

```
filterOffTarget(scores, min.score = 0.01, topN = 200,
    topN.OfftargetTotalScore = 20,
    annotateExon = TRUE, txdb, orgAnn,ignore.strand = TRUE,
    outputDir, oneFilePergRNA = FALSE,
    fetchSequence = TRUE, upstream = 200, downstream = 200, BSgenomeName,
    baseBeforegRNA = 4, baseAfterPAM = 3,
    featureWeightMatrixFile = system.file("extdata", "DoenchNBT2014.csv",
package = "CRISPRseek"),
    rule.set = c("Root_RuleSet1_2014", "Root_RuleSet2_2016", "CRISPRscan"),
    calculategRNAefficacyForOfftargets = TRUE)
```

Arguments

scores	a data frame output from getOfftargetScore. It contains strand (strand of the off target, + for plus and - for minus strand), chrom (chromosome of the off target), chromStart (start position of the off target), chromEnd (end position of the off target),name (gRNA name),gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), OffTargetSequence (the genomic sequence of the off target), n.mismatch (number of mismatches between the off target and the gRNA), forViewInUCSC (string for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score (score of the off target), mismatch.distance2PAM (a comma separated distances of all mismatches to PAM, e.g., 14,11 means one mismatch is 14 bp away from PAM and the other mismatch is 11 bp away from PAM), alignment (alignment between gRNA and off target, e.g., ...G..C.......... means that this off target aligns with gRNA except that G and C are mismatches),NGG (this off target contains canonical PAM or not, 1 for yes and 0 for no) mean.neighbor.distance.mismatch (mean distance between neighboring mismatches)
min.score	minimum score of an off target to included in the final output, default 0.5
topN	top N off targets to be included in the final output, default 100
topN.OfftargetTotalScore	
	top N off target used to calculate the total off target score, default 10
annotateExon	Choose whether or not to indicate whether the off target is inside an exon or not, default TRUE
txdb	TxDb object, for creating and using TxDb object, please refer to GenomicFeatures package. For a list of existing TxDb object, please search for annotation package starting with Txdb at http://www.bioconductor.org/packages/release/BiocViews.html#___Annotation such as TxDb.Rnorvegicus.UCSC.rn5.refGene for rat, TxDb.Mmusculus.UCSC.mm10.knownGene for mouse, TxDb.Hsapiens.UCSC.hg19.knownGene for human, TxDb.Dmelanogaster.UCSC.dm3.ensGene for Drosophila and TxDb.Celegans.UCSC.ce6.ensGene for C.elegans
orgAnn	organism annotation mapping such as org.Hs.egSYMBOL in org.Hs.eg.db package for human
ignore.strand	default to TRUE
outputDir	the directory where the off target analysis and reports will be written to
oneFilePergRNA	write to one file for each gRNA or not, default to FALSE
fetchSequence	Fetch flank sequence of off target or not, default TRUE
upstream	upstream offset from the off target start, default 200
downstream	downstream offset from the off target end, default 200
BSgenomeName	BSgenome object. Please refer to available.genomes in BSgenome package. For example, BSgenome.Hsapiens.UCSC.hg19 for hg19, BSgenome.Mmusculus.UCSC.mm10 for mm10, BSgenome.Celegans.UCSC.ce6 for ce6, BSgenome.Rnorvegicus.UCSC.rn5 for rn5, and BSgenome.Dmelanogaster.UCSC.dm3 for dm3
baseBeforegRNA	Number of bases before gRNA used for calculating gRNA efficiency, default 4
baseAfterPAM	Number of bases after PAM used for calculating gRNA efficiency, default 3
featureWeightMatrixFile	
	Feature weight matrix file used for calculating gRNA efficiency. By default DoenchNBT2014 weight matrix is used. To use alternative weight matrix file,

please input a csv file with first column containing significant features and the
second column containing the corresponding weights for the features. Please
see Doench et al., 2014 for details.

rule.set   Specify a rule set scoring system for calculating gRNA efficacy.

calculategRNAefficacyForOfftargets

Default to TRUE to output gRNA efficacy for offtargets as well as ontargets. Set
it to FALSE if only need gRNA efficacy calculated for ontargets only to speed up
the analysis. Please refer to https://support.bioconductor.org/p/133538/#133661
for potential use cases of offtarget efficacies.

Value

offtargets   a data frame with off target analysis results

summary   a data frame with summary of the off target analysis results

Author(s)

Lihua Julie Zhu

References

Doench JG, Hartenian E, Graham DB, Tothova Z, Hegde M, Smith I, Sullender M, Ebert BL,
Xavier RJ, Root DE. Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene
inactivation. Nat Biotechnol. 2014 Sep 3. doi: 10.1038 nbt.3026 Lihua Julie Zhu, Benjamin R.
Holmes, Neil Aronin and Michael Brodsky. CRISPRseek: a Bioconductor package to identify
target-specific guide RNAs for CRISPR-Cas9 genome-editing systems. Plos One Sept 23rd 2014

See Also

offTargetAnalysis

Examples

```
library(CRISPRseek)
library("BSgenome.Hsapiens.UCSC.hg19")
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
library(org.Hs.eg.db)
hitsFile <-  system.file("extdata", "hits.txt", package="CRISPRseek")
hits <- read.table(hitsFile, sep = "\t", header = TRUE,
    stringsAsFactors = FALSE)
featureVectors <- buildFeatureVectorForScoring(hits)
scores <- getOfftargetScore(featureVectors)
outputDir <- getwd()
results <- filterOffTarget(scores, BSgenomeName = Hsapiens,
    txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
     orgAnn = org.Hs.egSYMBOL, outputDir = outputDir,
    min.score = 0.1, topN = 10, topN.OfftargetTotalScore = 5)
results$offtargets
results$summary
```

---

findgRNAs   *Find potential gRNAs*

Description

Find potential gRNAs for an input file containing sequences in fasta format

Usage

```
findgRNAs(inputFilePath, baseEditing = FALSE, targetBase = "C", editingWindow = 4:8,
    format = "fasta", PAM = "NGG", PAM.size = 3,
    findPairedgRNAOnly = FALSE, annotatePaired = TRUE,
    paired.orientation = c("PAMout", "PAMin"), enable.multicore = FALSE,
    n.cores.max = 6, gRNA.pattern = "", gRNA.size = 20,
    overlap.gRNA.positions = c(17,18),
    primeEditing = FALSE,
        PBS.length = 13L,
        RT.template.length = 8:28,
        RT.template.pattern = "D$",
        corrected.seq,
        targeted.seq.length.change,
        bp.after.target.end = 15L,
        target.start,
        target.end,
        primeEditingPaired.output = "pairedgRNAsForPE.xls",
    min.gap = 0, max.gap = 20,
    pairOutputFile, name.prefix = "",
    featureWeightMatrixFile = system.file("extdata",
        "DoenchNBT2014.csv", package = "CRISPRseek"), baseBeforegRNA = 4,
        baseAfterPAM = 3, calculategRNAEfficacy = FALSE, efficacyFile,
    PAM.location = "3prime",
    rule.set = c("Root_RuleSet1_2014", "Root_RuleSet2_2016", "CRISPRscan"))
```

Arguments

inputFilePath	Sequence input file path or a DNAStringSet object that contains sequences to be searched for potential gRNAs
baseEditing	Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE, please set baseEditing = TRUE, targetBase and editingWidow accordingly.
targetBase	Applicable only when baseEditing is set to TRUE. It is used to indicate the target base for base editing systems, default to C for converting C to T in the CBE system. Please change it to A if you intend to use the ABE system.
editingWindow	Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window.

format	Format of the input file, fasta and fastq are supported, default fasta
PAM	protospacer-adjacent motif (PAM) sequence after the gRNA, default NGG
PAM.size	PAM length, default 3
findPairedgRNAOnly	
	Choose whether to only search for paired gRNAs in such an orientation that the first one is on minus strand called reverse gRNA and the second one is on plus strand called forward gRNA. TRUE or FALSE, default FALSE
annotatePaired	Indicate whether to output paired information, default TRUE
paired.orientation	
	PAMin orientation means the two adjacent PAMs on the sense and antisense strands face inwards towards each other like N21GG and CCN21 whereas PAMout orientation means they face away from each other like CCN21 and N21GG
enable.multicore	
	Indicate whether enable parallel processing, default FALSE. For super long sequences with lots of gRNAs, suggest set it to TRUE
n.cores.max	Indicating maximum number of cores to use in multi core mode, i.e., parallel processing, default 6. Please set it to 1 to disable multicore processing for small dataset.
gRNA.pattern	Regular expression or IUPAC Extended Genetic Alphabet to represent gRNA pattern, default is no restriction. To specify that the gRNA must start with GG for example, then set it to ^GG. Please see help(translatePattern) for a list of IUPAC Extended Genetic Alphabet.
gRNA.size	The size of the gRNA, default 20
overlap.gRNA.positions	
	The required overlap positions of gRNA and restriction enzyme cut site, default 17 and 18
primeEditing	Indicate whether to design gRNAs for prime editing. Default to FALSE. If true, please set PBS.length, RT.template.length, RT.template.pattern, targeted.seq.length.change, bp.after.target.end, target.start, and target.end accordingly
PBS.length	Applicable only when primeEditing is set to TRUE. It is used to specify the number of bases to ouput for primer binding site.
RT.template.length	
	Applicable only when primeEditing is set to TRUE. It is used to specify the number of bases required for RT template, default to 8 to 18. Please increase the length if the edit is large insertion. Only gRNAs with calculated RT.template.length falling into the specified range will be in the output. It is calculated as the following. RT.template.length = target.start – cut.start + (target.end - target.start) + targeted.seq.length.change + bp.after.target.end
RT.template.pattern	
	Applicable only when primeEditing is set to TRUE. It is used to specify the RT template sequence pattern, default to not ending with C according to https://doi.org/10.1038/s41586-019-1711-4
corrected.seq	Applicable only when primeEditing is set to TRUE. It is used to specify the mutated or inserted sequences after successful editing.

targeted.seq.length.change

    Applicable only when primeEditing is set to TRUE. It is used to specify the number of targeted sequence length change. Please set it to 0 for base changes, positive numbers for insersion, and negative number for deletion. For example, 10 means that the corrected sequence will have 10bp insertion, -10 means that the corrected sequence will have 10bp deletion, and 0 means only bases have been changed and the sequence length remains the same

bp.after.target.end

    Applicable only when primeEditing is set to TRUE. It is used to specify the number of bases to add after the target change end site as part of RT template. Please refer to RT.template.length for how this parameter influences the RT.template.length calculation which is used as a filtering criteria in pregRNA selection.

target.start   Applicable only when primeEditing is set to TRUE. It is used to specify the start location in the input sequence to make changes, which will be used to obtain the RT template sequence. Please also refer to RT.template.length for how this parameter influences the RT.template.length calculation which is used as a filtering criteria in pregRNA selection.

target.end   Applicable only when primeEditing is set to TRUE. It is used to specify the end location in the input sequnence to make changes, which will be used to obtain the RT template sequence. Please also refer to RT.template.length for how this parameter influences the RT.template.length calculation which is used as a filtering criteria in pregRNA selection.

primeEditingPaired.output

    Applicable only when primeEditing is set to TRUE. It is used to specify the file path to save pegRNA and the second gRNA with PBS, RT.template, gRNA sequences, default pairedgRNAsForPE.xls

min.gap   Minimum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 0

max.gap   Maximum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 20

pairOutputFile   The output file for writing paired gRNA information to

name.prefix   The prefix used when assign name to found gRNAs, default gRNA, short for guided RNA.

baseBeforegRNA   Number of bases before gRNA used for calculating gRNA efficiency, default 4 for spCas9 Please note, for PAM located on the 5 prime, need to specify the number of bases before the PAM sequence plus PAM size.

baseAfterPAM   Number of bases after PAM used for calculating gRNA efficiency, default 3 for spCas9 Please note, for PAM located on the 5 prime, need to include the length of the gRNA plus the extended sequence on the 3 prime

featureWeightMatrixFile

    Feature weight matrix file used for calculating gRNA efficiency. By default DoenchNBT2014 weight matrix is used. To use alternative weight matrix file, please input a csv file with first column containing significant features and the second column containing the corresponding weights for the features. Please see Doench et al., 2014 for details.

calculategRNAEfficacy
:   Default to FALSE, not to calculate gRNA efficacy

`efficacyFile`   File path to write gRNA efficacies

`PAM.location`   PAM location relative to gRNA. For example, spCas9 PAM is located on the 3 prime while cpf1 PAM is located on the 5 prime

`rule.set`   Specify a rule set scoring system for calculating gRNA efficacy.

Details

If users already has a fasta file that contains a set of potential gRNAs, then users can call filergRNAs directly although the easiest way is to call the one-stop-shopping function OffTargetAnalysis with findgRNAs set to FALSE.

Value

DNAStringSet consists of potential gRNAs that can be input to filtergRNAs function directly

Note

If the input sequence file contains multiple >300 bp sequences, suggest create one input file for each sequence and run the OffTargetAnalysis separately.

Author(s)

Lihua Julie Zhu

See Also

offTargetAnalysis

Examples

```
findgRNAs(inputFilePath = system.file("extdata",
    "inputseq.fa", package = "CRISPRseek"),
    pairOutputFile = "testpairedgRNAs.xls",
    findPairedgRNAOnly = TRUE)


##### predict gRNA efficacy using CRISPRscan
featureWeightMatrixFile <- system.file("extdata", "Morenos-Mateo.csv",
        package = "CRISPRseek")

findgRNAs(inputFilePath = system.file("extdata",
    "inputseq.fa", package = "CRISPRseek"),
    pairOutputFile = "testpairedgRNAs.xls",
    findPairedgRNAOnly = FALSE,
    calculategRNAEfficacy= TRUE,
    rule.set = "CRISPRscan",
    baseBeforegRNA = 6, baseAfterPAM = 6,
    featureWeightMatrixFile = featureWeightMatrixFile,
    efficacyFile = "testCRISPRscanEfficacy.xls"
```

```
   )
    findgRNAs(inputFilePath = system.file("extdata",
       "testCRISPRscan.fa", package = "CRISPRseek"),
       pairOutputFile = "testpairedgRNAs.xls",
       findPairedgRNAOnly = FALSE,
       calculategRNAEfficacy= TRUE,
       rule.set = "CRISPRscan",
       baseBeforegRNA = 6, baseAfterPAM = 6,
       featureWeightMatrixFile = featureWeightMatrixFile,
       efficacyFile = "testCRISPRscanEfficacy.xls"
    )

   findgRNAs(inputFilePath = system.file("extdata",
       "cpf1.fa", package = "CRISPRseek"),
       findPairedgRNAOnly=FALSE,
       pairOutputFile = "testpairedgRNAs-cpf1.xls",
       PAM="TTTN", PAM.location = "5prime", PAM.size = 4,
       overlap.gRNA.positions = c(19,23),
       baseBeforegRNA = 8, baseAfterPAM = 23,
       calculategRNAEfficacy= TRUE,
       featureWeightMatrixFile = system.file("extdata",
           "DoenchNBT2014.csv", package = "CRISPRseek"),
     efficacyFile = "testcpf1Efficacy.xls")

   findgRNAs(inputFilePath = system.file("extdata",
           "cpf1.fa", package = "CRISPRseek"),
           findPairedgRNAOnly=FALSE,
           pairOutputFile = "testpairedgRNAs-cpf1.xls",
           PAM="TTTN", PAM.location = "5prime", PAM.size = 4,
           overlap.gRNA.positions = c(19,23),
           baseBeforegRNA = 8, baseAfterPAM = 23,
           calculategRNAEfficacy= TRUE,
           featureWeightMatrixFile = system.file("extdata",
               "DoenchNBT2014.csv", package = "CRISPRseek"),
           efficacyFile = "testcpf1Efficacy.xls", baseEditing =  TRUE,
           editingWindow=20, targetBase = "X")

   findgRNAs(inputFilePath = system.file("extdata",
           "cpf1.fa", package = "CRISPRseek"),
           findPairedgRNAOnly=FALSE,
           pairOutputFile = "testpairedgRNAs-cpf1.xls",
           PAM="TTTN", PAM.location = "5prime", PAM.size = 4,
           overlap.gRNA.positions = c(19,23),
           baseBeforegRNA = 8, baseAfterPAM = 23,
           calculategRNAEfficacy= TRUE,
           featureWeightMatrixFile = system.file("extdata",
               "DoenchNBT2014.csv", package = "CRISPRseek"),
           efficacyFile = "testcpf1Efficacy.xls", baseEditing =  TRUE,
           editingWindow=20, targetBase = "C")

     inputSeq <-  DNAStringSet(paste(
"CCAGTTTGTGGATCCTGCTCTGGTGTCCTCCACACCAGAATCAGGGATCGAAAACTCA",
```

```
"TCAGTCGATGCGAGTCATCTAAATTCCGATCAATTTCACACTTTAAACG", sep =""))
    gRNAs <-  findgRNAs(inputFilePath =  inputSeq,
        pairOutputFile = "testpairedgRNAs1.xls",
        PAM.size = 3L,
        gRNA.size = 20L,
        overlap.gRNA.positions = c(17L,18L),
        PBS.length = 15,
        corrected.seq = "T",
        RT.template.pattern = "D$",
        RT.template.length = 8:30,
        targeted.seq.length.change = 0,
        bp.after.target.end = 15,
        target.start = 46,
        target.end = 46,
        paired.orientation = "PAMin", min.gap = 20, max.gap = 90,
        primeEditing = TRUE, findPairedgRNAOnly = TRUE)
```

---

getOfftargetScore   *Calculate score for each off target*

Description

Calculate score for each off target with given feature vectors and weights vector

Usage

```
getOfftargetScore(featureVectors,
    weights = c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445, 0.508,
    0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583))
```

Arguments

featureVectors   a data frame generated from buildFeatureVectorForScoring. It contains IsMis-
                 match.posX (Indicator variable indicating whether this position X is mismatch
                 or not, 1 means yes and 0 means not, X = 1- gRNA.size) representing all
                 positions in the gRNA), strand (strand of the off target, + for plus and - for
                 minus strand), chrom (chromosome of the off target), chromStart (start posi-
                 tion of the off target), chromEnd (end position of the off target), name (gRNA
                 name),gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), Off-
                 TargetSequence (the genomic sequence of the off target), n.mismatch (number
                 of mismatches between the off target and the gRNA), forViewInUCSC (string
                 for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score
                 (score of the off target), mismatch.distance2PAM (a comma separated distances
                 of all mismatches to PAM, e.g., 14,11 means one mismatch is 14 bp away from
                 PAM and the other mismatch is 11 bp away from PAM), alignment (alignment
                 between gRNA and off target, e.g., ...G..C.......... means that this off target
                 aligns with gRNA except that G and C are mismatches),NGG (this off target con-
                 tains canonical PAM or not, 1 for yes and 0 for no) mean.neighbor.distance.mismatch
                 (mean distance between neighboring mismatches)

weights   a numeric vector size of gRNA length, default c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445, 0.508, 0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583) which is used in Hsu et al., 2013 cited in the reference section

Details

score is calculated using the weights and algorithm by Hsu et al., 2013 cited in the reference section

Value

a data frame containing strand (strand of the match, + for plus and - for minus strand), chrom (chromosome of the off target), chromStart (start position of the off target), chromEnd (end position of the off target),name (gRNA name), gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), OffTargetSequence (the genomic sequence of the off target), n.mismatch (number of mismatches between the off target and the gRNA), forViewInUCSC (string for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score (score of the off target), mismatch.distance2PAM (a comma separated distances of all mismatches to PAM, e.g., 14,11 means one mismatch is 14 bp away from PAM and the other mismatch is 11 bp away from PAM), alignment (alignment between gRNA and off target, e.g., ...G..C.......... means that this off target aligns with gRNA except that G and C are mismatches), NGG (this off target contains canonical PAM or not, 1 for yes and 0 for no) mean.neighbor.distance.mismatch (mean distance between neighboring mismatches)

Author(s)

Lihua Julie Zhu

References

Patrick D Hsu, David A Scott, Joshua A Weinstein, F Ann Ran, Silvana Konermann, Vineeta Agarwala, Yinqing Li, Eli J Fine, Xuebing Wu, Ophir Shalem, Thomas J Cradick, Luciano A Marraffini, Gang Bao & Feng Zhang (2013) DNA targeting specificity of rNA-guided Cas9 nucleases. Nature Biotechnology 31:827-834

See Also

offTargetAnalysis

Examples

```
hitsFile <-  system.file("extdata", "hits.txt",
    package = "CRISPRseek")
hits <- read.table(hitsFile, sep = "\t", header = TRUE,
    stringsAsFactors = FALSE)
featureVectors <- buildFeatureVectorForScoring(hits)
getOfftargetScore(featureVectors)
```

---

isPatternUnique   *Output whether the input patterns occurs only once in the sequence*

Description

Input a sequence and a list of patterns and determine if the patterns occurs only once in the sequence. Used for determining whether a RE site in gRNA also occurs in the flanking region.

Usage

```
isPatternUnique(seq, patterns)
```

Arguments

seq   flanking sequence of a gRNA

patterns   patterns as DNAStringSet, such as a list of RE sites

Value

returns a character vectors containing the uniqueness of each pattern/RE site

Author(s)

Lihua Julie Zhu

Examples

```
seq <- "TGGATTGTATAATCAGCATGGATTTGGAAC"
patterns <- DNAStringSet(c("TGG", "TGGA", "TGGATA", "TTGGAAC", ""))
isPatternUnique(seq, patterns)
isPatternUnique(seq)
isPatternUnique(patterns)
```

---

offTargetAnalysis   *Design of target-specific guide RNAs for CRISPR-Cas9 system in one function*

Description

Design of target-specific guide RNAs (gRNAs) and predict relative indel fequencies for CRISPR-Cas9 system by automatically calling findgRNAs, filtergRNAs, searchHits, buildFeatureVector-ForScoring, getOfftargetScore, filterOfftarget, calculating gRNA cleavage efficiency, and predict gRNA efficacy, indels and their frequencies.

Usage

```
offTargetAnalysis(inputFilePath, format = "fasta", header = FALSE,
    gRNAoutputName, findgRNAs = TRUE,
    exportAllgRNAs = c("all", "fasta", "genbank", "no"),
    findgRNAsWithREcutOnly = FALSE,
    REpatternFile = system.file("extdata", "NEBenzymes.fa",
        package = "CRISPRseek"), minREpatternSize = 4,
    overlap.gRNA.positions = c(17, 18), findPairedgRNAOnly = FALSE,
    annotatePaired = TRUE, paired.orientation = c("PAMout","PAMin"),
    enable.multicore = FALSE, n.cores.max = 6,
    min.gap = 0, max.gap = 20, gRNA.name.prefix = "", PAM.size = 3,
    gRNA.size = 20,  PAM = "NGG",  BSgenomeName, chromToSearch = "all",
    chromToExclude = c("chr17_ctg5_hap1","chr4_ctg9_hap1", "chr6_apd_hap1",
"chr6_cox_hap2", "chr6_dbb_hap3", "chr6_mann_hap4", "chr6_mcf_hap5","chr6_qbl_hap6",
"chr6_ssto_hap7"),
    max.mismatch = 3, PAM.pattern = "NNG$|NGN$", allowed.mismatch.PAM = 1,
    gRNA.pattern = "",
    baseEditing = FALSE, targetBase = "C", editingWindow = 4:8,
    editingWindow.offtargets = 4:8,
    primeEditing = FALSE,
        PBS.length = 13L,
        RT.template.length = 8:28,
        RT.template.pattern = "D$",
        corrected.seq,
        targeted.seq.length.change,
        bp.after.target.end = 15L,
        target.start,
        target.end,
    primeEditingPaired.output = "pairedgRNAsForPE.xls",
    min.score = 0, topN = 1000,
    topN.OfftargetTotalScore = 10, annotateExon = TRUE,
    txdb, orgAnn, ignore.strand = TRUE,
    outputDir, fetchSequence = TRUE, upstream = 200, downstream = 200,
    upstream.search = 0, downstream.search = 0,
    weights = c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445, 0.508,
    0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583),
    baseBeforegRNA = 4, baseAfterPAM = 3,
    featureWeightMatrixFile = system.file("extdata", "DoenchNBT2014.csv",
package = "CRISPRseek"), useScore = TRUE, useEfficacyFromInputSeq = FALSE,
    outputUniqueREs = TRUE, foldgRNAs = FALSE,
    gRNA.backbone =
"GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU",
    temperature = 37,
    overwrite = FALSE,
    scoring.method = c("Hsu-Zhang", "CFDscore"),
        subPAM.activity = hash( AA =0,
          AC =   0,
          AG = 0.259259259,
```

```
        AT = 0,
        CA = 0,
        CC = 0,
        CG = 0.107142857,
        CT = 0,
        GA = 0.069444444,
        GC = 0.022222222,
        GG = 1,
        GT = 0.016129032,
        TA = 0,
        TC = 0,
        TG = 0.038961039,
        TT = 0),
    subPAM.position = c(22, 23),
    PAM.location = "3prime",
    rule.set = c("Root_RuleSet1_2014", "Root_RuleSet2_2016", "CRISPRscan"),
    calculategRNAefficacyForOfftargets = TRUE,
    mismatch.activity.file = system.file("extdata",
        "NatureBiot2016SuppTable19DoenchRoot.csv",
        package = "CRISPRseek"),
    predIndelFreq = FALSE,
    predictIndelFreq.onTargetOnly = TRUE,
    method.indelFreq = "Lindel",
    baseBeforegRNA.indelFreq = 13,
    baseAfterPAM.indelFreq = 24
  )
```

Arguments

inputFilePath	Sequence input file path or a DNAStringSet object that contains sequences to be searched for potential gRNAs
format	Format of the input file, fasta, fastq and bed are supported, default fasta
header	Indicate whether the input file contains header, default FALSE, only applies to bed format
gRNAoutputName	Specify the name of the gRNA outupt file when inputFilePath is DNAStringSet object instead of file path
findgRNAs	Indicate whether to find gRNAs from the sequences in the input file or skip the step of finding gRNAs, default TRUE. Set it to FALSE if the input file contains user selected gRNAs plus PAM already.
exportAllgRNAs	Indicate whether to output all potential gRNAs to a file in fasta format, genbank format or both. Default to both.
findgRNAsWithREcutOnly	
	Indicate whether to find gRNAs overlap with restriction enzyme recognition pattern
REpatternFile	File path containing restriction enzyme cut patterns
minREpatternSize	
	Minimum restriction enzyme recognition pattern length required for the enzyme pattern to be searched for, default 4

overlap.gRNA.positions

        The required overlap positions of gRNA and restriction enzyme cut site, default 17 and 18

findPairedgRNAOnly

        Choose whether to only search for paired gRNAs in such an orientation that the first one is on minus strand called reverse gRNA and the second one is on plus strand called forward gRNA. TRUE or FALSE, default FALSE

annotatePaired Indicate whether to output paired information, default TRUE

paired.orientation

        PAMin orientation means the two adjacent PAMs on the sense and antisense strands face inwards towards each other like N21GG and CCN21 whereas PAMout orientation means they face away from each other like CCN21 and N21GG

min.gap   Minimum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 0

enable.multicore

        Indicate whether enable parallel processing, default FALSE. For super long sequences with lots of gRNAs, suggest set it to TRUE

n.cores.max   Indicating maximum number of cores to use in multi core mode, i.e., parallel processing, default 6. Please set it to 1 to disable multicore processing for small dataset.

max.gap   Maximum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 20

gRNA.name.prefix

        The prefix used when assign name to found gRNAs, default gRNA, short for guided RNA.

PAM.size   PAM length, default 3

gRNA.size   The size of the gRNA, default 20

PAM   PAM sequence after the gRNA, default NGG

BSgenomeName BSgenome object. Please refer to available.genomes in BSgenome package. For example, BSgenome.Hsapiens.UCSC.hg19 for hg19, BSgenome.Mmusculus.UCSC.mm10 for mm10, BSgenome.Celegans.UCSC.ce6 for ce6, BSgenome.Rnorvegicus.UCSC.rn5 for rn5, BSgenome.Drerio.UCSC.danRer7 for Zv9, and BSgenome.Dmelanogaster.UCSC.dm3 for dm3

chromToSearch Specify the chromosome to search, default to all, meaning search all chromosomes. For example, chrX indicates searching for matching in chromosome X only

chromToExclude Specify the chromosome not to search. If specified as "", meaning to search chromosomes specified by chromToSearch. By default, to exclude haplotype blocks from offtarget search in hg19, i.e., chromToExclude = c("chr17_ctg5_hap1","chr4_ctg9_hap1", "chr6_apd_hap1", "chr6_cox_hap2", "chr6_dbb_hap3", "chr6_mann_hap4", "chr6_mcf_hap5","chr6_qbl "chr6_ssto_hap7")

max.mismatch Maximum mismatch allowed in off target search, default 3. Warning: will be considerably slower if set > 3

PAM.pattern   Regular expression of protospacer-adjacent motif (PAM), default NNG\$|NGN\$ for spCas9. For cpf1, ^TTTN since it is a 5 prime PAM sequence

`allowed.mismatch.PAM`

Maximum number of mismatches allowed in the PAM sequence for offtarget search, default to 1 to allow NGN and NNG PAM pattern for offtarget identification.

`gRNA.pattern`   Regular expression or IUPAC Extended Genetic Alphabet to represent gRNA pattern, default is no restriction. To specify that the gRNA must start with GG for example, then set it to ^GG. Please see help(translatePattern) for a list of IUPAC Extended Genetic Alphabet.

`baseEditing`   Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE, please set baseEditing = TRUE, targetBase and editingWidow accordingly.

`targetBase`   Applicable only when baseEditing is set to TRUE. It is used to indicate the target base for base editing systems, default to C for converting C to T in the CBE system. Please change it to A if you intend to use the ABE system.

`editingWindow`   Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window.

`editingWindow.offtargets`

Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window to consider for the offtargets search only, default to 4 to 8 (1 means the most distal site from the 3' PAM, the most proximla site from the 5' PAM), which is for the original CBE system. Please change it accordingly if the system you use have a different editing window, or you would like to include offtargets with the target base in a larger editing window.

`primeEditing`   Indicate whether to design gRNAs for prime editing. Default to FALSE. If true, please set PBS.length, RT.template.length, RT.template.pattern, targeted.seq.length.change, bp.after.target.end, target.start, and target.end accordingly

`PBS.length`   Applicable only when primeEditing is set to TRUE. It is used to specify the number of bases to ouput for primer binding site.

`RT.template.length`

Applicable only when primeEditing is set to TRUE. It is used to specify the number of bases required for RT template, default to 8 to 18. Please increase the length if the edit is large insertion. Only gRNAs with calculated RT.template.length falling into the specified range will be in the output. It is calculated as the following. RT.template.length = target.start – cut.start + (target.end - target.start) + targeted.seq.length.change + bp.after.target.end

`RT.template.pattern`

Applicable only when primeEditing is set to TRUE. It is used to specify the RT template sequence pattern, default to not ending with C according to https://doi.org/10.1038/s41586-019-1711-4

`corrected.seq`   Applicable only when primeEditing is set to TRUE. It is used to specify the mutated or inserted sequences after successful editing.

`targeted.seq.length.change`

Applicable only when primeEditing is set to TRUE. It is used to specify the number of targeted sequence length change. Please set it to 0 for base changes, positive numbers for insertion, and negative number for deletion. For example,

10 means that the corrected sequence will have 10bp insertion, -10 means that the corrected sequence will have 10bp deletion, and 0 means only bases have been changed and the sequence length remains the same

bp.after.target.end

Applicable only when primeEditing is set to TRUE. It is used to specify the number of bases to add after the target change end site as part of RT template. Please refer to RT.template.length for how this parameter influences the RT.template.length calculation which is used as a filtering criteria in pregRNA selection.

target.start   Applicable only when primeEditing is set to TRUE. It is used to specify the start location in the input sequence to make changes, which will be used to obtain the RT template sequence. Please also refer to RT.template.length for how this parameter influences the RT.template.length calculation which is used as a filtering criteria in pregRNA selection.

target.end   Applicable only when primeEditing is set to TRUE. It is used to specify the end location in the input sequnence to make changes, which will be used to obtain the RT template sequence. Please also refer to RT.template.length for how this parameter influences the RT.template.length calculation which is used as a filtering criteria in pregRNA selection.

primeEditingPaired.output

Applicable only when primeEditing is set to TRUE. It is used to specify the file path to save pegRNA and the second gRNA with PBS, RT.template, gRNA sequences, default pairedgRNAsForPE.xls

min.score   minimum score of an off target to included in the final output, default 0

topN   top N off targets to be included in the final output, default 1000

topN.OfftargetTotalScore

top N off target used to calculate the total off target score, default 10

annotateExon   Choose whether or not to indicate whether the off target is inside an exon or not, default TRUE

txdb   TxDb object, for creating and using TxDb object, please refer to GenomicFeatures package. For a list of existing TxDb object, please search for annotation package starting with Txdb at http://www.bioconductor.org/packages/release/BiocViews.html#___Annota such as TxDb.Rnorvegicus.UCSC.rn5.refGene for rat, TxDb.Mmusculus.UCSC.mm10.knownGene for mouse, TxDb.Hsapiens.UCSC.hg19.knownGene for human, TxDb.Dmelanogaster.UCSC.dm3.ensGen for Drosophila and TxDb.Celegans.UCSC.ce6.ensGene for C.elegans

orgAnn   organism annotation mapping such as org.Hs.egSYMBOL in org.Hs.eg.db package for human

ignore.strand   default to TRUE when annotating to gene

outputDir   the directory where the off target analysis and reports will be written to

fetchSequence   Fetch flank sequence of off target or not, default TRUE

upstream   upstream offset from the off target start, default 200

downstream   downstream offset from the off target end, default 200

upstream.search

upstream offset from the bed input starts to search for gRNAs, default 0

downstream.search

                downstream offset from the bed input ends to search for gRNAs, default 0

weights   Applicable only when scoring.method is set to Hsu-Zhang a numeric vector size of gRNA length, default c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445, 0.508, 0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583) which is used in Hsu et al., 2013 cited in the reference section

baseBeforegRNA Number of bases before gRNA used for calculating gRNA efficiency, default 4 Please note, for PAM located on the 5 prime, need to specify the number of bases before the PAM sequence plus PAM size.

baseAfterPAM   Number of bases after PAM used for calculating gRNA efficiency, default 3 for spCas9 Please note, for PAM located on the 5 prime, need to include the length of the gRNA plus the extended sequence on the 3 prime

featureWeightMatrixFile

                Feature weight matrix file used for calculating gRNA efficiency. By default DoenchNBT2014 weight matrix is used. To use alternative weight matrix file, please input a csv file with first column containing significant features and the second column containing the corresponding weights for the features. Please see Doench et al., 2014 for details.

useScore   Default TRUE, display in gray scale with the darkness indicating the gRNA efficacy. The taller bar shows the Cas9 cutting site. If set to False, efficacy will not show. Instead, gRNAs in plus strand will be colored red and gRNAs in negative strand will be colored green.

useEfficacyFromInputSeq

                Default FALSE. If set to TRUE, summary file will contain gRNA efficacy calculated from input sequences instead of from off-target analysis. Set it to TRUE if the input sequence is from a different species than the one used for off-target analysis.

outputUniqueREs

                Default TRUE. If set to TRUE, summary file will contain REs unique to the cleavage site within 100 or 200 bases surrounding the gRNA sequence.

foldgRNAs   Default FALSE. If set to TRUE, summary file will contain minimum free energy of the secondary structure of gRNA with gRNA backbone from GeneRfold package provided that GeneRfold package has been installed.

gRNA.backbone   gRNA backbone constant region sequence. Default to the sequence in Sp gRNA backbone.

temperature   temperature in celsius. Default to 37 celsius.

overwrite   overwrite the existing files in the output directory or not, default FALSE

scoring.method Indicates which method to use for offtarget cleavage rate estimation, currently two methods are supported, Hsu-Zhang and CFDscore

subPAM.activity

                Applicable only when scoring.method is set to CFDscore A hash to represent the cleavage rate for each alternative sub PAM sequence relative to preferred PAM sequence

subPAM.position

                Applicable only when scoring.method is set to CFDscore The start and end positions of the sub PAM. Default to 22 and 23 for spCas9 with 20bp gRNA and NGG as preferred PAM. For Cpf1, it could be c(1,2).

PAM.location   PAM location relative to gRNA. For example, default to 3prime for spCas9
                   PAM. Please set to 5prime for cpf1 PAM since it's PAM is located on the 5
                   prime end

rule.set   Specify a rule set scoring system for calculating gRNA efficacy. Please note
                   that Root_RuleSet2_2016 requires the following python packages with specified
                   verion and python 2.7. 1. scikit-learn 0.16.1 2. pickle 3. pandas 4. numpy 5.
                   scipy

calculategRNAefficacyForOfftargets
                   Default to TRUE to output gRNA efficacy for offtargets as well as ontargets. Set
                   it to FALSE if only need gRNA efficacy calculated for ontargets only to speed up
                   the analysis. Please refer to https://support.bioconductor.org/p/133538/#133661
                   for potential use cases of offtarget efficacies.

mismatch.activity.file
                   Applicable only when scoring.method is set to CFDscore A comma separated
                   (csv) file containing the cleavage rates for all possible types of single nucleotide
                   mismatche at each position of the gRNA. By default, using the supplemental
                   Table 19 from Doench et al., Nature Biotechnology 2016

predIndelFreq   Default to FALSE. Set it to TRUE to output the predicted indels and their fre-
                   quencies.

predictIndelFreq.onTargetOnly
                   Default to TRUE, indicating that indels and their frequencies will be predicted
                   for ontargets only. Usually, researchers are only interested in predicting the edit-
                   ing outcome for the ontargets since any editing in the offtargets are unwanted.
                   Set it to FALSE if you are interested in predicting indels and their frequencies
                   for offtargets. It will take longer time to run if you set it to FALSE.

method.indelFreq
                   Currently only Lindel method has been implemented. Please let us know if you
                   think additional methods should be made available. Lindel is compatible with
                   both Python2.7 and Python3.5 or higher. Please type help(predictRelativeFreqIndels)
                   to get more details.

baseBeforegRNA.indelFreq
                   Default to 13 for Lindel method.

baseAfterPAM.indelFreq
                   Default to 24 for Lindel method.

Value

Four tab delimited files are generated in the output directory: OfftargetAnalysis.xls (detailed infor-
mation of off targets), Summary.xls (summary of the gRNAs), REcutDetails.xls (restriction enzyme
cut sites of each gRNA), and pairedgRNAs.xls (potential paired gRNAs)

Author(s)

Lihua Julie Zhu

References

Patrick D Hsu, David A Scott, Joshua A Weinstein, F Ann Ran, Silvana Konermann, Vineeta Agarwala, Yinqing Li, Eli J Fine, Xuebing Wu, Ophir Shalem, Thomas J Cradick, Luciano A Marraffini, Gang Bao & Feng Zhang (2013) DNA targeting specificity of rNA-guided Cas9 nucleases. Nature Biotechnology 31:827-834

Doench JG, Hartenian E, Graham DB, Tothova Z, Hegde M, Smith I, Sullender M, Ebert BL, Xavier RJ, Root DE. Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. Nat Biotechnol. 2014 Sep 3. doi: 10.1038 nbt.3026

Lihua Julie Zhu, Benjamin R. Holmes, Neil Aronin and Michael Brodsky. CRISPRseek: a Bioconductor package to identify target-specific guide RNAs for CRISPR-Cas9 genome-editing systems. Plos One Sept 23rd 2014

Moreno-Mateos, M., Vejnar, C., Beaudoin, J. et al. CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo. Nat Methods 12, 982–988 (2015) doi:10.1038/nmeth.3543

Doench JG et al., Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nature Biotechnology Jan 18th 2016

Anzalone et al., Search-and-replace genome editing without double-strand breaks or donor DNA. Nature October 2019 https://www.nature.com/articles/s41586-019-1711-4

Wei Chen, Aaron McKenna, Jacob Schreiber et al., Massively parallel profiling and predictive modeling of the outcomes of CRISPR/Cas9-mediated double-strand break repair, Nucleic Acids Research, Volume 47, Issue 15, 05 September 2019, Pages 7989–8003, https://doi.org/10.1093/nar/gkz487

See Also

CRISPRseek

Examples

```
library(CRISPRseek)
library("BSgenome.Hsapiens.UCSC.hg19")
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
library(org.Hs.eg.db)
outputDir <- getwd()
inputFilePath <- system.file("extdata", "inputseq.fa",
         package = "CRISPRseek")
REpatternFile <- system.file("extdata", "NEBenzymes.fa",
         package = "CRISPRseek")
results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly = TRUE,
         REpatternFile = REpatternFile, findPairedgRNAOnly = FALSE,
         annotatePaired = FALSE,
         BSgenomeName = Hsapiens, chromToSearch = "chrX",
         txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
    orgAnn = org.Hs.egSYMBOL, max.mismatch = 1,
         outputDir = outputDir, overwrite = TRUE)

    #### predict indels and their frequecies for target sites

    if (interactive())
    {
```

```
      results <- offTargetAnalysis(inputFilePath,findgRNAsWithREcutOnly = TRUE,
        findPairedgRNAOnly = FALSE,
        annotatePaired = FALSE,
        BSgenomeName = Hsapiens, chromToSearch = "chrX",
        txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
   orgAnn = org.Hs.egSYMBOL, max.mismatch = 1,
        outputDir = outputDir, overwrite = TRUE,
        predIndelFreq=TRUE, predictIndelFreq.onTargetOnly= TRUE)

      names(results$indelFreq)
      head(results$indelFreq[[1]])
   ### save the indel frequences to tab delimited files, one file for each target/offtarget site.
   mapply(write.table, results$indelFreq, file=paste0(names(results$indelFreq), '.xls'), sep = "\t", row.name
   }
   #### predict gRNA efficacy using CRISPRscan
   featureWeightMatrixFile <- system.file("extdata", "Morenos-Mateo.csv",
        package = "CRISPRseek")

   results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly = TRUE,
        REpatternFile = REpatternFile, findPairedgRNAOnly = FALSE,
        annotatePaired = FALSE,
        BSgenomeName = Hsapiens, chromToSearch = "chrX",
        txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
        orgAnn = org.Hs.egSYMBOL, max.mismatch = 1,
        rule.set = "CRISPRscan",
        baseBeforegRNA = 6, baseAfterPAM = 6,
        featureWeightMatrixFile = featureWeightMatrixFile,
        outputDir = outputDir, overwrite = TRUE)

   ######## PAM is on the 5 prime side
   results <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly =  FALSE,
        REpatternFile = REpatternFile, findPairedgRNAOnly = FALSE,
        annotatePaired = FALSE,
        BSgenomeName = Hsapiens, chromToSearch = "chrX",
        txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
        orgAnn = org.Hs.egSYMBOL, max.mismatch = 4,
        outputDir = outputDir, overwrite = TRUE, PAM.location = "5prime",
        PAM = "TGT", PAM.pattern = "^T[A|G]N", allowed.mismatch.PAM = 2,
        subPAM.position = c(1,2))

    results1 <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly =  FALSE,
            REpatternFile = REpatternFile, findPairedgRNAOnly = FALSE,
            annotatePaired = FALSE,
            BSgenomeName = Hsapiens, chromToSearch = "chrX",
            txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
            orgAnn = org.Hs.egSYMBOL, max.mismatch = 4,
            outputDir = outputDir, overwrite = TRUE, PAM.location = "5prime",
            PAM = "TGT", PAM.pattern = "^T[A|G]N", allowed.mismatch.PAM = 2,
        subPAM.position = c(1,2), baseEditing = TRUE, editingWindow =20, targetBase = "G")

   results.testBE <- offTargetAnalysis(inputFilePath, findgRNAsWithREcutOnly =  FALSE,
            REpatternFile = REpatternFile, findPairedgRNAOnly = FALSE,
            annotatePaired = FALSE,
```

```
                BSgenomeName = Hsapiens, chromToSearch = "chrX",
                txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
                orgAnn = org.Hs.egSYMBOL, max.mismatch = 4,
                outputDir = outputDir, overwrite = TRUE, PAM.location = "5prime",
                PAM = "TGT", PAM.pattern = "^T[A|G]N", allowed.mismatch.PAM = 2,
                subPAM.position = c(1,2), baseEditing = TRUE,
                editingWindow = 10:20, targetBase = "A")

        inputFilePath <- DNAStringSet(paste(
"CCAGTTTGTGGATCCTGCTCTGGTGTCCTCCACACCAGAATCAGGGATCGAAAA",
"CTCATCAGTCGATGCGAGTCATCTAAATTCCGATCAATTTCACACTTTAAACG", sep =""))
        names(inputFilePath) <- "testPE"
        results3 <- offTargetAnalysis(inputFilePath,
            gRNAoutputName = "testPEgRNAs",
            BSgenomeName = Hsapiens, chromToSearch = "chrX",
            txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
            orgAnn = org.Hs.egSYMBOL, max.mismatch = 1,
            outputDir = outputDir, overwrite = TRUE,
            PAM.size = 3L,
            gRNA.size = 20L,
            overlap.gRNA.positions = c(17L,18L),
            PBS.length = 15,
            corrected.seq = "T",
            RT.template.pattern = "D$",
            RT.template.length = 8:30,
            targeted.seq.length.change = 0,
            bp.after.target.end = 15,
            target.start = 20,
            target.end = 20,
            paired.orientation = "PAMin", min.gap = 20, max.gap = 90,
            primeEditing = TRUE, findPairedgRNAOnly = TRUE)
```

---

offTargetAnalysisWithoutBSgenome

Design of target-specific guide RNAs for CRISPR-Cas9 system in one function without BSgenome

---

Description

Design of target-specific guide RNAs (gRNAs) for CRISPR-Cas9 system without BSgenome by automatically calling findgRNAs, filtergRNAs, searchHits, buildFeatureVectorForScoring, getOff-targetScore, filterOfftarget, calculating gRNA cleavage efficiency and generate reports.

Usage

```
offTargetAnalysisWithoutBSgenome(inputFilePath, format = "fasta", header = FALSE,
    gRNAoutputName, findgRNAs = TRUE,
    exportAllgRNAs = c("all", "fasta", "genbank", "no"),
    findgRNAsWithREcutOnly = FALSE,
```

```
    REpatternFile = system.file("extdata", "NEBenzymes.fa",
        package = "CRISPRseek"), minREpatternSize = 4,
    overlap.gRNA.positions = c(17, 18), findPairedgRNAOnly = FALSE,
    annotatePaired = TRUE, paired.orientation = c("PAMout","PAMin"),
    enable.multicore = FALSE, n.cores.max = 6,
    min.gap = 0, max.gap = 20, gRNA.name.prefix = "", PAM.size = 3,
    gRNA.size = 20,  PAM = "NGG",  BSgenomeName, chromToSearch = "all",
    chromToExclude = c("chr17_ctg5_hap1","chr4_ctg9_hap1", "chr6_apd_hap1",
"chr6_cox_hap2", "chr6_dbb_hap3", "chr6_mann_hap4", "chr6_mcf_hap5","chr6_qbl_hap6",
"chr6_ssto_hap7"),
    max.mismatch = 3, PAM.pattern = "NNG$|NGN$", allowed.mismatch.PAM = 1,
    gRNA.pattern = "",
    baseEditing = FALSE, targetBase = "C", editingWindow = 4:8,
    editingWindow.offtargets = 4:8,
    primeEditing = FALSE,
        PBS.length = 13L,
        RT.template.length = 8:28,
        RT.template.pattern = "D$",
        corrected.seq,
        targeted.seq.length.change,
        bp.after.target.end = 15L,
        target.start,
        target.end,
    primeEditingPaired.output = "pairedgRNAsForPE.xls",
    min.score = 0, topN = 1000,
    topN.OfftargetTotalScore = 10, annotateExon = TRUE,
    txdb, orgAnn, ignore.strand = TRUE,
    outputDir, fetchSequence = TRUE, upstream = 200, downstream = 200,
    upstream.search = 0, downstream.search = 0,
    weights = c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445, 0.508,
    0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583),
    baseBeforegRNA = 4, baseAfterPAM = 3,
    featureWeightMatrixFile = system.file("extdata", "DoenchNBT2014.csv",
package = "CRISPRseek"), useScore = TRUE, useEfficacyFromInputSeq = FALSE,
    outputUniqueREs = TRUE, foldgRNAs = FALSE,
    gRNA.backbone =
"GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUU",
    temperature = 37,
    overwrite = FALSE,
    scoring.method = c("Hsu-Zhang", "CFDscore"),
        subPAM.activity = hash( AA =0,
          AC =   0,
          AG = 0.259259259,
          AT = 0,
          CA = 0,
          CC = 0,
          CG = 0.107142857,
          CT = 0,
```

```
            GA = 0.069444444,
            GC = 0.022222222,
            GG = 1,
            GT = 0.016129032,
            TA = 0,
            TC = 0,
            TG = 0.038961039,
            TT = 0),
       subPAM.position = c(22, 23),
       PAM.location = "3prime",
       rule.set = c("Root_RuleSet1_2014", "Root_RuleSet2_2016", "CRISPRscan"),
       mismatch.activity.file = system.file("extdata",
           "NatureBiot2016SuppTable19DoenchRoot.csv",
           package = "CRISPRseek"),
       useBSgenome = FALSE, genomeSeqFile,
       predIndelFreq = FALSE,
       predictIndelFreq.onTargetOnly = TRUE,
       method.indelFreq = "Lindel",
       baseBeforegRNA.indelFreq = 13,
       baseAfterPAM.indelFreq = 24
     )
```

Arguments

inputFilePath	Sequence input file path or a DNAStringSet object that contains sequences to be searched for potential gRNAs
format	Format of the input file, fasta, fastq and bed are supported, default fasta
header	Indicate whether the input file contains header, default FALSE, only applies to bed format
gRNAoutputName	Specify the name of the gRNA outupt file when inputFilePath is DNAStringSet object instead of file path
findgRNAs	Indicate whether to find gRNAs from the sequences in the input file or skip the step of finding gRNAs, default TRUE. Set it to FALSE if the input file contains user selected gRNAs plus PAM already.
exportAllgRNAs	Indicate whether to output all potential gRNAs to a file in fasta format, genbank format or both. Default to both.
findgRNAsWithREcutOnly	
	Indicate whether to find gRNAs overlap with restriction enzyme recognition pattern
REpatternFile	File path containing restriction enzyme cut patterns
minREpatternSize	
	Minimum restriction enzyme recognition pattern length required for the enzyme pattern to be searched for, default 4
overlap.gRNA.positions	
	The required overlap positions of gRNA and restriction enzyme cut site, default 17 and 18

findPairedgRNAOnly

    Choose whether to only search for paired gRNAs in such an orientation that the first one is on minus strand called reverse gRNA and the second one is on plus strand called forward gRNA. TRUE or FALSE, default FALSE

annotatePaired   Indicate whether to output paired information, default TRUE

paired.orientation

    PAMin orientation means the two adjacent PAMs on the sense and antisense strands face inwards towards each other like N21GG and CCN21 whereas PAMout orientation means they face away from each other like CCN21 and N21GG

min.gap   Minimum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 0

enable.multicore

    Indicate whether enable parallel processing, default FALSE. For super long sequences with lots of gRNAs, suggest set it to TRUE

n.cores.max   Indicating maximum number of cores to use in multi core mode, i.e., parallel processing, default 6. Please set it to 1 to disable multicore processing for small dataset.

max.gap   Maximum distance between two oppositely oriented gRNAs to be valid paired gRNAs. Default 20

gRNA.name.prefix

    The prefix used when assign name to found gRNAs, default gRNA, short for guided RNA.

PAM.size   PAM length, default 3

gRNA.size   The size of the gRNA, default 20

PAM   PAM sequence after the gRNA, default NGG

BSgenomeName   BSgenome object. Please refer to available.genomes in BSgenome package. For example, BSgenome.Hsapiens.UCSC.hg19 for hg19, BSgenome.Mmusculus.UCSC.mm10 for mm10, BSgenome.Celegans.UCSC.ce6 for ce6, BSgenome.Rnorvegicus.UCSC.rn5 for rn5, BSgenome.Drerio.UCSC.danRer7 for Zv9, and BSgenome.Dmelanogaster.UCSC.dm3 for dm3

chromToSearch   Specify the chromosome to search, default to all, meaning search all chromosomes. For example, chrX indicates searching for matching in chromosome X only

chromToExclude   Specify the chromosome not to search. If specified as "", meaning to search chromosomes specified by chromToSearch. By default, to exclude haplotype blocks from offtarget search in hg19, i.e., chromToExclude = c("chr17_ctg5_hap1","chr4_ctg9_hap1", "chr6_apd_hap1", "chr6_cox_hap2", "chr6_dbb_hap3", "chr6_mann_hap4", "chr6_mcf_hap5","chr6_qbl "chr6_ssto_hap7")

max.mismatch   Maximum mismatch allowed in off target search, default 3. Warning: will be considerably slower if set > 3

PAM.pattern   Regular expression of protospacer-adjacent motif (PAM), default NNG\$|NGN\$ for spCas9. For cpf1, ^TTTN since it is a 5 prime PAM sequence

allowed.mismatch.PAM

    Maximum number of mismatches allowed in the PAM sequence for offtarget search, default to 1 for NNG or NGN PAM pattern for offtarget finding.

gRNA.pattern   Regular expression or IUPAC Extended Genetic Alphabet to represent gRNA
                    pattern, default is no restriction. To specify that the gRNA must start with GG
                    for example, then set it to ^GG. Please see help(translatePattern) for a list of
                    IUPAC Extended Genetic Alphabet.

baseEditing   Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE,
                    please set baseEditing = TRUE, targetBase and editingWidow accordingly.

targetBase   Applicable only when baseEditing is set to TRUE. It is used to indicate the
                    target base for base editing systems, default to C for converting C to T in the
                    CBE system. Please change it to A if you intend to use the ABE system.

editingWindow   Applicable only when baseEditing is set to TRUE. It is used to indicate the ef-
                    fective editing window, default to 4 to 8 which is for the original CBE system.
                    Please change it accordingly if the system you use have a different editing win-
                    dow.

editingWindow.offtargets
                    Applicable only when baseEditing is set to TRUE. It is used to indicate the
                    effective editing window to consider for the offtargets search only, default to 4 to
                    8 (1 means the most distal site from the 3' PAM, the most proximla site from the
                    5' PAM), which is for the original CBE system. Please change it accordingly if
                    the system you use have a different editing window, or you would like to include
                    offtargets with the target base in a larger editing window.

primeEditing   Indicate whether to design gRNAs for prime editing. Default to FALSE. If true,
                    please set PBS.length, RT.template.length, RT.template.pattern, targeted.seq.length.change,
                    bp.after.target.end, target.start, and target.end accordingly

PBS.length   Applicable only when primeEditing is set to TRUE. It is used to specify the
                    number of bases to ouput for primer binding site.

RT.template.length
                    Applicable only when primeEditing is set to TRUE. It is used to specify the
                    number of bases required for RT template, default to 8 to 18. Please increase the
                    length if the edit is large insertion. Only gRNAs with calculated RT.template.length
                    falling into the specified range will be in the output. It is calculated as the fol-
                    lowing. RT.template.length = target.start – cut.start + (target.end - target.start) +
                    targeted.seq.length.change + bp.after.target.end

RT.template.pattern
                    Applicable only when primeEditing is set to TRUE. It is used to specify the RT
                    template sequence pattern, default to not ending with C according to https://doi.org/10.1038/s41586-
                    019-1711-4

corrected.seq   Applicable only when primeEditing is set to TRUE. It is used to specify the
                    mutated or inserted sequences after successful editing.

targeted.seq.length.change
                    Applicable only when primeEditing is set to TRUE. It is used to specify the
                    number of targeted sequence length change. Please set it to 0 for base changes,
                    positive numbers for insersion, and negative number for deletion. For example,
                    10 means that the corrected sequence will have 10bp insertion, -10 means that
                    the corrected sequence will have 10bp deletion, and 0 means only bases have
                    been changed and the sequence length remains the same

bp.after.target.end

 Applicable only when primeEditing is set to TRUE. It is used to specify the
 number of bases to add after the target change end site as part of RT tem-
 plate. Please refer to RT.template.length for how this parameter influences the
 RT.template.length calculation which is used as a filtering criteria in pregRNA
 selection.

target.start Applicable only when primeEditing is set to TRUE. It is used to specify the
 start location in the input sequence to make changes, which will be used to
 obtain the RT template sequence. Please also refer to RT.template.length for
 how this parameter influences the RT.template.length calculation which is used
 as a filtering criteria in pregRNA selection.

target.end Applicable only when primeEditing is set to TRUE. It is used to specify the
 end location in the input sequnence to make changes, which will be used to
 obtain the RT template sequence. Please also refer to RT.template.length for
 how this parameter influences the RT.template.length calculation which is used
 as a filtering criteria in pregRNA selection.

primeEditingPaired.output

 Applicable only when primeEditing is set to TRUE. It is used to specify the
 file path to save pegRNA and the second gRNA with PBS, RT.template, gRNA
 sequences, default pairedgRNAsForPE.xls

min.score minimum score of an off target to included in the final output, default 0

topN top N off targets to be included in the final output, default 1000

topN.OfftargetTotalScore

 top N off target used to calculate the total off target score, default 10

annotateExon Choose whether or not to indicate whether the off target is inside an exon or not,
 default TRUE

txdb TxDb object, for creating and using TxDb object, please refer to GenomicFea-
 tures package. For a list of existing TxDb object, please search for annotation
 package starting with Txdb at http://www.bioconductor.org/packages/release/BiocViews.html#___Annota
 such as TxDb.Rnorvegicus.UCSC.rn5.refGene for rat, TxDb.Mmusculus.UCSC.mm10.knownGene
 for mouse, TxDb.Hsapiens.UCSC.hg19.knownGene for human, TxDb.Dmelanogaster.UCSC.dm3.ensGer
 for Drosophila and TxDb.Celegans.UCSC.ce6.ensGene for C.elegans

orgAnn organism annotation mapping such as org.Hs.egSYMBOL in org.Hs.eg.db pack-
 age for human

ignore.strand default to TRUE when annotating to gene

outputDir the directory where the off target analysis and reports will be written to

fetchSequence Fetch flank sequence of off target or not, default TRUE

upstream upstream offset from the off target start, default 200

downstream downstream offset from the off target end, default 200

upstream.search

 upstream offset from the bed input starts to search for gRNAs, default 0

downstream.search

 downstream offset from the bed input ends to search for gRNAs, default 0

weights   Applicable only when scoring.method is set to Hsu-Zhang a numeric vector size
                   of gRNA length, default c(0, 0, 0.014, 0, 0, 0.395, 0.317, 0, 0.389, 0.079, 0.445,
                   0.508, 0.613, 0.851, 0.732, 0.828, 0.615, 0.804, 0.685, 0.583) which is used in
                   Hsu et al., 2013 cited in the reference section

baseBeforegRNA   Number of bases before gRNA used for calculating gRNA efficiency, default
                   4 Please note, for PAM located on the 5 prime, need to specify the number of
                   bases before the PAM sequence plus PAM size.

baseAfterPAM   Number of bases after PAM used for calculating gRNA efficiency, default 3 for
                   spCas9 Please note, for PAM located on the 5 prime, need to include the length
                   of the gRNA plus the extended sequence on the 3 prime

featureWeightMatrixFile
                   Feature weight matrix file used for calculating gRNA efficiency. By default
                   DoenchNBT2014 weight matrix is used. To use alternative weight matrix file,
                   please input a csv file with first column containing significant features and the
                   second column containing the corresponding weights for the features. Please
                   see Doench et al., 2014 for details.

useScore   Default TRUE, display in gray scale with the darkness indicating the gRNA
                   efficacy. The taller bar shows the Cas9 cutting site. If set to False, efficacy
                   will not show. Instead, gRNAs in plus strand will be colored red and gRNAs in
                   negative strand will be colored green.

useEfficacyFromInputSeq
                   Default FALSE. If set to TRUE, summary file will contain gRNA efficacy cal-
                   culated from input sequences instead of from off-target analysis. Set it to TRUE
                   if the input sequence is from a different species than the one used for off-target
                   analysis.

outputUniqueREs
                   Default TRUE. If set to TRUE, summary file will contain REs unique to the
                   cleavage site within 100 or 200 bases surrounding the gRNA sequence.

foldgRNAs   Default FALSE. If set to TRUE, summary file will contain minimum free en-
                   ergy of the secondary structure of gRNA with gRNA backbone from GeneRfold
                   package provided that GeneRfold package has been installed.

gRNA.backbone   gRNA backbone constant region sequence. Default to the sequence in Sp gRNA
                   backbone.

temperature   temperature in celsius. Default to 37 celsius.

overwrite   overwrite the existing files in the output directory or not, default FALSE

scoring.method   Indicates which method to use for offtarget cleavage rate estimation, currently
                   two methods are supported, Hsu-Zhang and CFDscore

subPAM.activity
                   Applicable only when scoring.method is set to CFDscore A hash to represent
                   the cleavage rate for each alternative sub PAM sequence relative to preferred
                   PAM sequence

subPAM.position
                   Applicable only when scoring.method is set to CFDscore The start and end po-
                   sitions of the sub PAM. Default to 22 and 23 for spCas9 with 20bp gRNA and
                   NGG as preferred PAM. For Cpf1, it could be c(1,2).

`PAM.location`   PAM location relative to gRNA. For example, default to 3prime for spCas9
                      PAM. Please set to 5prime for cpf1 PAM since it's PAM is located on the 5
                      prime end

`rule.set`   Specify a rule set scoring system for calculating gRNA efficacy. Please note
                      that Root_RuleSet2_2016 requires the following python packages with specified
                      verion and python 2.7. 1. scikit-learn 0.16.1 2. pickle 3. pandas 4. numpy 5.
                      scipy

`mismatch.activity.file`
                      Applicable only when scoring.method is set to CFDscore A comma separated
                      (csv) file containing the cleavage rates for all possible types of single nucleotide
                      mismatche at each position of the gRNA. By default, using the supplemental
                      Table 19 from Doench et al., Nature Biotechnology 2016

`useBSgenome`   Specify whether BSgenome is avaialbe for searching for gRNA and offtargets,
                      default to FALSE. If set it to TRUE, the results should be the same as when
                      using offtargetAnalysis function.

`genomeSeqFile`   Specify the genome sequence file in fasta format. It is only applicable and re-
                      quired when useBSgenome is set to FALSE.

`predIndelFreq`   Default to FALSE. Set it to TRUE to output the predicted indels and their fre-
                      quencies.

`predictIndelFreq.onTargetOnly`
                      Default to TRUE, indicating that indels and their frequencies will be predicted
                      for ontargets only. Usually, researchers are only interested in predicting the edit-
                      ing outcome for the ontargets since any editing in the offtargets are unwanted.
                      Set it to FALSE if you are interested in predicting indels and their frequencies
                      for offtargets. It will take longer time to run if you set it to FALSE.

`method.indelFreq`
                      Currently only Lindel method has been implemented. Please let us know if you
                      think additional methods should be made available. Lindel is compatible with
                      both Python2.7 and Python3.5 or higher. Please type help(predictRelativeFreqIndels)
                      to get more details.

`baseBeforegRNA.indelFreq`
                      Default to 13 for Lindel method.

`baseAfterPAM.indelFreq`
                      Default to 24 for Lindel method.

Value

Four tab delimited files are generated in the output directory: OfftargetAnalysis.xls (detailed infor-
mation of off targets), Summary.xls (summary of the gRNAs), REcutDetails.xls (restriction enzyme
cut sites of each gRNA), and pairedgRNAs.xls (potential paired gRNAs)

Author(s)

Lihua Julie Zhu

References

Patrick D Hsu, David A Scott, Joshua A Weinstein, F Ann Ran, Silvana Konermann, Vineeta Agarwala, Yinqing Li, Eli J Fine, Xuebing Wu, Ophir Shalem, Thomas J Cradick, Luciano A Marraffini, Gang Bao & Feng Zhang (2013) DNA targeting specificity of rNA-guided Cas9 nucleases. Nature Biotechnology 31:827-834

Doench JG, Hartenian E, Graham DB, Tothova Z, Hegde M, Smith I, Sullender M, Ebert BL, Xavier RJ, Root DE. Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation. Nat Biotechnol. 2014 Sep 3. doi: 10.1038 nbt.3026

Lihua Julie Zhu, Benjamin R. Holmes, Neil Aronin and Michael Brodsky. CRISPRseek: a Bioconductor package to identify target-specific guide RNAs for CRISPR-Cas9 genome-editing systems. Plos One Sept 23rd 2014

Moreno-Mateos, M., Vejnar, C., Beaudoin, J. et al. CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo. Nat Methods 12, 982–988 (2015) doi:10.1038/nmeth.3543

Doench JG et al., Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nature Biotechnology Jan 18th 2016

Anzalone et al., Search-and-replace genome editing without double-strand breaks or donor DNA. Nature October 2019 https://www.nature.com/articles/s41586-019-1711-4

Wei Chen, Aaron McKenna, Jacob Schreiber et al., Massively parallel profiling and predictive modeling of the outcomes of CRISPR/Cas9-mediated double-strand break repair, Nucleic Acids Research, Volume 47, Issue 15, 05 September 2019, Pages 7989–8003, https://doi.org/10.1093/nar/gkz487

See Also

CRISPRseek

Examples

```
library(CRISPRseek)
inputFilePath <- system.file("extdata", "inputseqWithoutBSgenome.fa",
            package = "CRISPRseek")
########## genomeSeq.fasta contains the genomic sequence in fasta format for gRNA and offtarget search###########
        genomeSeqFile <- system.file("extdata", "genomeSeq.fasta",
            package = "CRISPRseek")
        library(hash)
        subPAM.activity <- hash(AA = 0, AC = 0, AG = 0.259259259,
            AT = 0, CA = 0, CC = 0, CG = 0.107142857, CT = 0, GA = 0.069444444,
            GC = 0.022222222, GG = 1, GT = 0.016129032, TA = 0, TC = 0,
            TG = 0.038961039, TT = 0)
        featureWeightMatrixFile <- system.file("extdata", "Morenos-Mateo.csv",
            package = "CRISPRseek")
results <- offTargetAnalysisWithoutBSgenome(inputFilePath = inputFilePath,
            format = "fasta",
            useBSgenome = FALSE,
            genomeSeqFile = genomeSeqFile,
            findgRNAs = TRUE,
            exportAllgRNAs = "fasta",
            fetchSequence = FALSE,
            findgRNAsWithREcutOnly = FALSE,
```

```
            findPairedgRNAOnly = FALSE,
            annotatePaired = FALSE,
    max.mismatch = 1,
            annotateExon = FALSE,
            scoring.method = "CFDscore",
            min.score = 0.01,
            PAM = "NGG",
            PAM.pattern <- "NNN",
            rule.set = "CRISPRscan",
            featureWeightMatrixFile = featureWeightMatrixFile,
            subPAM.activity  = subPAM.activity,
            outputDir = "gRNAoutput-CRISPRseek-CRISPRscan-CFDscore", overwrite = TRUE)
```

---

predictRelativeFreqIndels

Predict insertions and deletions induced by CRISPR/Cas9 editing

---

Description

Predict insertions and deletions, and associated reletive frequecies induced by CRISPR/Cas9 editing

Usage

```
predictRelativeFreqIndels(extendedSequence, method = "Lindel")
```

Arguments

extendedSequence

A vector of DNA sequences of length 60bp. It consists 30bp before the cut site and 30bp after the cut site.

method   the prediction method. default to Lindel. Currently only Lindel method are implemented.

Details

Predict relative indel frequency around target sites of CRISPR/Cas9 system. Currently only Lindel method using logistic regression is implemented in CRISPRseek.

Lindel is compatible with both Python2.7 and Python3.5 or higher.

By default, reticulate uses the version of Python found on your PATH (i.e. Sys.which("python")).

Use the function use_python in reticulate library to set the python path to a specific version. For example, use_python('/opt/anaconda3/lib/python3.7')

This function implements the Lindel method

Value

A list with the same length as the input extendedSequence.

Each list item either contains a warning message, or a predicted fraction of frameshift in the mutational outcomes plus a data frame with three columns.

The three columns are the alignment of predicted indel sequence to the original unedited sequence, predicted indel frequency, and the location of the predicted indels. The warning message for the Lindel method is as follows.

Warning: No PAM sequence is identified. Please check your sequence and try again.

Author(s)

Hui Mao and Lihua Julie Zhu

References

Wei Chen, Aaron McKenna, Jacob Schreiber et al., Massively parallel profiling and predictive modeling of the outcomes of CRISPR/Cas9-mediated double-strand break repair, Nucleic Acids Research, Volume 47, Issue 15, 05 September 2019, Pages 7989–8003, https://doi.org/10.1093/nar/gkz487

Examples

```
extendedSequence <- c("AAA", "TAACGTTATCAACGCCTATATTAAAGCGACCGTCGGTTGAACTGCGTGGATCAATGCGTC")
if (interactive())
    indelFreq <- predictRelativeFreqIndels(extendedSequence, method = "Lindel")
```

---

searchHits   *Search for off targets in a sequence as DNAString*

Description

Search for off targets for given gRNAs, sequence and maximum mismatches

Usage

```
searchHits(gRNAs, seqs, seqname, max.mismatch = 3, PAM.size = 3,
                gRNA.size = 20, PAM = "NGG", PAM.pattern = "NNN$",
                allowed.mismatch.PAM = 2, PAM.location = "3prime",
                outfile,
                baseEditing = FALSE, targetBase = "C", editingWindow = 4:8)
```

Arguments

gRNAs	DNAStringSet object containing a set of gRNAs. Please note the sequences must contain PAM appended after gRNAs, e.g., ATCGAAATTCGAGCCAATC-CCGG where ATCGAAATTCGAGCCAATCC is the gRNA and CGG is the PAM
seqs	DNAString object containing a DNA sequence.
seqname	Specify the name of the sequence
max.mismatch	Maximum mismatch allowed in off target search, default 3. Warning: will be considerably slower if it is set to greater than 3
PAM.size	Size of PAM, default 3
gRNA.size	Size of gRNA, default 20
PAM	PAM as regular expression for appending to the gRNA, default NGG for Sp-Cas9, change to TTTN for cpf1.
PAM.pattern	Regular expression of PAM, default N[A|G]G\$ for spCas9. For cpf1, ^TTTN since it is a 5 prime PAM sequence
allowed.mismatch.PAM	
	Maximum number of mismatches allowed in the offtargets comparing to the PAM sequence. Default to 2 for NGG PAM
PAM.location	PAM location relative to gRNA. For example, spCas9 PAM is located on the 3 prime while cpf1 PAM is located on the 5 prime
outfile	File path to temporarily store the search results
baseEditing	Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE, please set baseEditing = TRUE, targetBase and editingWidow accordingly.
targetBase	Applicable only when baseEditing is set to TRUE. It is used to indicate the target base for base editing systems, default to C for converting C to T in the CBE system. Please change it to A if you intend to use the ABE system.
editingWindow	Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window to consider for the offtargets search only, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window, or you would like to include offtargets with the target base in a larger editing window.

Value

a data frame contains IsMismatch.posX (indicator variable indicating whether this position X is mismatch or not, 1 means yes and 0 means not, X = 1 to gRNA.size) representing all positions in the gRNA),strand (strand of the match, + for plus and - for minus strand), chrom (chromosome of the off target), chromStart (start position of the off target), chromEnd (end position of the off target),name (gRNA name), gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), OffTarget-Sequence (the genomic sequence of the off target), n.mismatch (number of mismatches between the off target and the gRNA), forViewInUCSC (string for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score (set to 100, and will be updated in getOfftargetScore)

Author(s)

Lihua Julie Zhu

See Also

offTargetAnalysis

Examples

```
 if(interactive())
 {
    all.gRNAs <- findgRNAs(inputFilePath =
        system.file("extdata", "inputseq.fa", package = "CRISPRseek"),
        pairOutputFile = "pairedgRNAs.xls",
findPairedgRNAOnly = TRUE)

    library("BSgenome.Hsapiens.UCSC.hg19")
    ### for speed reason, use max.mismatch = 0 for finding all targets with
    ### all variants of PAM
    hits <- searchHits(all.gRNAs[1], BSgenomeName = Hsapiens,
        max.mismatch = 0, chromToSearch = "chrX")
    colnames(hits)

    ### test PAM located at 5 prime
    all.gRNAs <- findgRNAs(inputFilePath =
            system.file("extdata", "inputseq.fa", package = "CRISPRseek"),
            pairOutputFile = "pairedgRNAs.xls",
            findPairedgRNAOnly = FALSE,
            PAM = "TGT", PAM.location = "5prime")

    library("BSgenome.Hsapiens.UCSC.hg19")
        ### for speed reason, use max.mismatch = 0 for finding all targets with
        ### all variants of PAM
    hits <- searchHits(all.gRNAs[1], BSgenomeName = Hsapiens, PAM.size = 3,
        max.mismatch = 0, chromToSearch = "chrX", PAM.location = "5prime",
        PAM = "^T[A|G]N", allowed.mismatch.PAM = 2)
    colnames(hits)
 }
```

---

searchHits2   *Search for off targets*

Description

Search for off targets for given gRNAs, BSgenome and maximum mismatches

Usage

```
searchHits2(gRNAs, BSgenomeName, chromToSearch = "all", chromToExclude = "",
    max.mismatch = 3,
    PAM.size = 3, gRNA.size = 20, PAM = "NGG", PAM.pattern = "N[A|G]G$",
    allowed.mismatch.PAM = 1, PAM.location = "3prime",
    baseEditing = FALSE, targetBase = "C", editingWindow = 4:8)
```

Arguments

gRNAs	DNAStringSet object containing a set of gRNAs. Please note the sequences must contain PAM appended after gRNAs, e.g., ATCGAAATTCGAGCCAATC-CCGG where ATCGAAATTCGAGCCAATCC is the gRNA and CGG is the PAM
BSgenomeName	BSgenome object. Please refer to available.genomes in BSgenome package. For example, BSgenome.Hsapiens.UCSC.hg19 for hg19, BSgenome.Mmusculus.UCSC.mm10 for mm10, BSgenome.Celegans.UCSC.ce6 for ce6, BSgenome.Rnorvegicus.UCSC.rn5 for rn5, and BSgenome.Dmelanogaster.UCSC.dm3 for dm3
chromToSearch	Specify the chromosome to search, default to all, meaning search all chromosomes. For example, chrX indicates searching for matching in chromosome X only
chromToExclude	Specify the chromosome not to search, default to none, meaning to search chromosomes specified by chromToSearch. For example, to exclude haplotype blocks from offtarget search in hg19, set chromToExclude to c(""chr17_ctg5_hap1","chr4_ctg9_hap1", "chr6_apd_hap1", "chr6_cox_hap2", "chr6_dbb_hap3", "chr6_mann_hap4", "chr6_mcf_hap5","chr6_qbl "chr6_ssto_hap7")
max.mismatch	Maximum mismatch allowed in off target search, default 3. Warning: will be considerably slower if it is set to greater than 3
PAM.size	Size of PAM, default 3
gRNA.size	Size of gRNA, default 20
PAM	Regular expression of protospacer-adjacent motif (PAM), default NGG for spCas9. For cpf1, ^TTTN
PAM.pattern	Regular expression of PAM, default N[A|G]G$ for spCas9. For cpf1, ^TTTN since it is a 5 prime PAM sequence
allowed.mismatch.PAM	
	Number of degenerative bases in the PAM sequence, default to 1 for N[A|G]G PAM
PAM.location	PAM location relative to gRNA. For example, spCas9 PAM is located on the 3 prime while cpf1 PAM is located on the 5 prime
baseEditing	Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE, please set baseEditing = TRUE, targetBase and editingWidow accordingly.
targetBase	Applicable only when baseEditing is set to TRUE. It is used to indicate the target base for base editing systems, default to C for converting C to T in the CBE system. Please change it to A if you intend to use the ABE system.
editingWindow	Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window to consider for the offtargets search only, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window, or you would like to include offtargets with the target base in a larger editing window.

Value

a data frame contains IsMismatch.posX (indicator variable indicating whether this position X is mismatch or not, 1 means yes and 0 means not, X = 1 to gRNA.size) representing all positions in the

gRNA),strand (strand of the match, + for plus and - for minus strand), chrom (chromosome of the off target), chromStart (start position of the off target), chromEnd (end position of the off target),name (gRNA name), gRNAPlusPAM (gRNA sequence with PAM sequence concatenated), OffTarget-Sequence (the genomic sequence of the off target), n.mismatch (number of mismatches between the off target and the gRNA), forViewInUCSC (string for viewing in UCSC genome browser, e.g., chr14:31665685-31665707), score (set to 100, and will be updated in getOfftargetScore)

Author(s)

Lihua Julie Zhu

See Also

offTargetAnalysis

Examples

```
    all.gRNAs <- findgRNAs(inputFilePath =
        system.file("extdata", "inputseq.fa", package = "CRISPRseek"),
        pairOutputFile = "pairedgRNAs.xls",
findPairedgRNAOnly = TRUE)

    library("BSgenome.Hsapiens.UCSC.hg19")
    ### for speed reason, use max.mismatch = 0 for finding all targets with
    ### all variants of PAM
    hits <- searchHits2(all.gRNAs[1], BSgenomeName = Hsapiens,
        max.mismatch = 0, chromToSearch = "chrX")
    colnames(hits)

    ### test PAM located at 5 prime
    all.gRNAs <- findgRNAs(inputFilePath =
            system.file("extdata", "inputseq.fa", package = "CRISPRseek"),
            pairOutputFile = "pairedgRNAs.xls",
            findPairedgRNAOnly = FALSE,
            PAM = "TGT", PAM.location = "5prime")

    library("BSgenome.Hsapiens.UCSC.hg19")
        ### for speed reason, use max.mismatch = 0 for finding all targets with
        ### all variants of PAM
    hits <- searchHits2(all.gRNAs[1], BSgenomeName = Hsapiens, PAM.size = 3,
        max.mismatch = 0, chromToSearch = "chrX", PAM.location = "5prime",
        PAM = "NGG",
        PAM.pattern = "^T[A|G]N", allowed.mismatch.PAM = 2)
    colnames(hits)
```

---

translatePattern   *translate pattern from IUPAC Extended Genetic Alphabet to regular expression*

Description

translate pattern containing the IUPAC nucleotide ambiguity codes to regular expression. For example,Y->[C|T], R-> [A|G], S-> [G|C], W-> [A|T], K-> [T|U|G], M-> [A|C], B-> [C|G|T], D-> [A|G|T], H-> [A|C|T], V-> [A|C|G] and N-> [A|C|T|G].

Usage

```
translatePattern(pattern)
```

Arguments

pattern   a character vector with the IUPAC nucleotide ambiguity codes

Value

a character vector with the pattern represented as regular expression

Author(s)

Lihua Julie Zhu

Examples

```
pattern1 <- "AACCNWMK"
translatePattern(pattern1)
```

---

uniqueREs   *Output restriction enzymes that recognize only the gRNA cleavage sites*

Description

For each identified gRNA, output restriction enzymes that recognize only the gRNA cleavage sites.

Usage

```
uniqueREs(REcutDetails, summary, offTargets, scanUpstream = 100,
    scanDownstream = 100, BSgenomeName)
```

Arguments

REcutDetails   REcutDetails stored in the REcutDetails.xls

summary   summary stored in the summary.xls

offTargets   offTargets stored in the offTargets.xls

scanUpstream   upstream offset from the gRNA start, default 100

scanDownstream downstream offset from the gRNA end, default 100

BSgenomeName   BSgenome object. Please refer to available.genomes in BSgenome package. For
                      example, BSgenome.Hsapiens.UCSC.hg19 for hg19, BSgenome.Mmusculus.UCSC.mm10
                      for mm10, BSgenome.Celegans.UCSC.ce6 for ce6, BSgenome.Rnorvegicus.UCSC.rn5
                      for rn5, BSgenome.Drerio.UCSC.danRer7 for Zv9, and BSgenome.Dmelanogaster.UCSC.dm3
                      for dm3

Value

returns the RE sites that recognize only the gRNA cleavage sites for each gRNA.

Author(s)

Lihua Julie Zhu

Examples

```
library("BSgenome.Hsapiens.UCSC.hg19")
load(system.file("extdata", "ForTestinguniqueREs.RData",
        package = "CRISPRseek"))
uniqueREs(results$REcutDetails, results$summary, results$offtarget,
scanUpstream = 50,
    scanDownstream = 50, BSgenomeName = Hsapiens)
```

---

writeHits   *Write the hits of sequence search from a sequence to a file*

Description

write the hits of sequence search from a sequence instead of BSgenome to a file, internal function
used by searchHits

Usage

```
writeHits(gRNA, seqname, matches, strand, file, gRNA.size = 20L,
    PAM = "NGG", PAM.pattern = "N[A|G]G$", max.mismatch = 4L,
    chrom.len, append = FALSE, PAM.location = "3prime",
    PAM.size = 3L, allowed.mismatch.PAM = 1L,
    seqs,
  baseEditing = FALSE, targetBase = "C", editingWindow = 4:8)
```

Arguments

gRNA   DNAString object with gRNA sequence with PAM appended immediately af-
              ter,e.g., ACGTACGTACGTACTGACGTCGG with 20bp gRNA sequence plus
              3bp PAM sequence CGG

seqname   sequence name as character

matches   XStringViews object storing matched chromosome locations

strand	strand of the match, + for plus strand and - for minus strand
file	file path where the hits is written to
gRNA.size	gRNA size, default 20
PAM	PAM as regular expression for appending to the gRNA, default NGG for Sp-Cas9, change to TTTN for cpf1.
PAM.pattern	PAM as regular expression for filtering the hits, default N[A|G]G\$ for spCas9. For cpf1, ^TTTN since it is a 5 prime PAM sequence.
max.mismatch	maximum mismatch allowed within the gRNA (excluding PAM portion) for filtering the hits, default 4
chrom.len	length of the matched chromosome
append	TRUE if append to existing file, false if start a new file
PAM.location	PAM location relative to gRNA. For example, spCas9 PAM is located on the 3 prime while cpf1 PAM is located on the 5 prime
PAM.size	Size of PAM, default 3
allowed.mismatch.PAM	
	Maximum number of mismatches allowed in the offtargets comparing to the PAM sequence. Default to 1 for NGG PAM
seqs	DNAString object containing a DNA sequence.
baseEditing	Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE, please set baseEditing = TRUE, targetBase and editingWidow accordingly.
targetBase	Applicable only when baseEditing is set to TRUE. It is used to indicate the target base for base editing systems, default to C for converting C to T in the CBE system. Please change it to A if you intend to use the ABE system.
editingWindow	Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window to consider for the offtargets search only, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window, or you would like to include offtargets with the target base in a larger editing window.

Value

results are saved in the file specified by file

Author(s)

Lihua Julie Zhu

References

http://bioconductor.org/packages/2.8/bioc/vignettes/BSgenome/inst/doc/ GenomeSearching.pdf

See Also

offTargetAnalysis

Examples

```
if(interactive())
{
    gRNAPlusPAM <- DNAString("ACGTACGTACGTACTGACGTCGG")
    x <- DNAString("AAGCGCGATATGACGTACGTACGTACTGACGTCGG")
    chrom.len <- nchar(as.character(x))
    m <- matchPattern(gRNAPlusPAM, x)
    names(m) <- "testing"
    writeHits(gRNA = gRNAPlusPAM, seqname = "chr1",
        matches = m, strand = "+", file = "exampleWriteHits.txt",
        chrom.len = chrom.len, append = FALSE)
}
```

---

writeHits2	*Write the hits of sequence search to a file*

Description

write the hits of sequence search to a file, internal function used by searchHits

Usage

```
writeHits2(gRNA, seqname, matches, strand, file, gRNA.size = 20,
    PAM = "NGG", PAM.pattern = "N[A|G]G$",
    max.mismatch = 4, chrom.len, append = FALSE,
    PAM.location = "3prime", PAM.size = 3,
    allowed.mismatch.PAM = 1L,
    BSgenomeName, baseEditing = FALSE, targetBase = "C", editingWindow = 4:8)
```

Arguments

gRNA	DNAString object with gRNA sequence with PAM appended immediately after,e.g., ACGTACGTACGTACTGACGTCGG with 20bp gRNA sequence plus 3bp PAM sequence CGG
seqname	chromosome name as character, e.g., chr1
matches	XStringViews object storing matched chromosome locations
strand	strand of the match, + for plus strand and - for minus strand
file	file path where the hits is written to
gRNA.size	gRNA size, default 20
PAM	PAM as regular expression for filtering the hits, default NGG for spCas9. For cpf1, TTTN.
PAM.pattern	Regular expression of protospacer-adjacent motif (PAM), default N[A|G]G$ for spCas9. For cpf1, ^TTTN since it is a 5 prime PAM sequence
max.mismatch	maximum mismatch allowed within the gRNA (excluding PAM portion) for filtering the hits, default 4

chrom.len	length of the matched chromosome
append	TRUE if append to existing file, false if start a new file
PAM.location	PAM location relative to gRNA. For example, spCas9 PAM is located on the 3 prime while cpf1 PAM is located on the 5 prime
PAM.size	Size of PAM, default 3
allowed.mismatch.PAM	
	Number of degenerative bases in the PAM sequence, default to 1 for N[A|G]G PAM
BSgenomeName	BSgenome object. Please refer to available.genomes in BSgenome package. For example, BSgenome.Hsapiens.UCSC.hg19 for hg19, BSgenome.Mmusculus.UCSC.mm10 for mm10, BSgenome.Celegans.UCSC.ce6 for ce6, BSgenome.Rnorvegicus.UCSC.rn5 for rn5, BSgenome.Drerio.UCSC.danRer7 for Zv9, and BSgenome.Dmelanogaster.UCSC.dm3 for dm3
baseEditing	Indicate whether to design gRNAs for base editing. Default to FALSE If TRUE, please set baseEditing = TRUE, targetBase and editingWidow accordingly.
targetBase	Applicable only when baseEditing is set to TRUE. It is used to indicate the target base for base editing systems, default to C for converting C to T in the CBE system. Please change it to A if you intend to use the ABE system.
editingWindow	Applicable only when baseEditing is set to TRUE. It is used to indicate the effective editing window to consider for the offtargets search only, default to 4 to 8 which is for the original CBE system. Please change it accordingly if the system you use have a different editing window, or you would like to include offtargets with the target base in a larger editing window.

Value

results are saved in the file specified by file

Author(s)

Lihua Julie Zhu

References

http://bioconductor.org/packages/2.8/bioc/vignettes/BSgenome/inst/doc/ GenomeSearching.pdf

See Also

offTargetAnalysis

Examples

```
library("BSgenome.Hsapiens.UCSC.hg19")
gRNAPlusPAM <- DNAString("ACGTACGTACGTACTGACGTCGG")
x <- DNAString("AAGCGCGATATGACGTACGTACGTACTGACGTCGG")
chrom.len <- nchar(as.character(x))
m <- matchPattern(gRNAPlusPAM, x)
names(m) <- "testing"
```

```
writeHits2(gRNA = gRNAPlusPAM, seqname = "chr1",
    PAM = "NGG", PAM.pattern = "NNN$", allowed.mismatch.PAM = 2,
    matches = m, strand = "+", file = "exampleWriteHits.txt",
    chrom.len = chrom.len, append = FALSE, BSgenomeName = Hsapiens)
```

Index