# Package 'CRISPRseek'

November 13, 2024

**Type** Package

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

**Version** 1.46.0

**Date** 2022-06-21

**Encoding** UTF-8

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**Depends** R (>= 3.5.0), BiocGenerics, Biostrings

**Imports** parallel, data.table, seqinr, S4Vectors (>= 0.9.25), IRanges, BSgenome, hash, methods,reticulate,rhdf5,XVector, DelayedArray, GenomeInfoDb, GenomicRanges, dplyr, keras, mltools

**Suggests** RUnit, BiocStyle, BSgenome.Hsapiens.UCSC.hg19, TxDb.Hsapiens.UCSC.hg19.knownGene, org.Hs.eg.db, BSgenome.Mmusculus.UCSC.mm10, TxDb.Mmusculus.UCSC.mm10.knownGene, org.Mm.eg.db, lattice, MASS, tensorflow, testthat

**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)

**LazyData** yes

**biocViews** ImmunoOncology, GeneRegulation, SequenceMatching, CRISPR

**RoxygenNote** 7.1.2

**NeedsCompilation** no

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

**git_branch** RELEASE_3_20

# Contents

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

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### 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, searching 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 directory: 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 each one (respectively) is inside an exon or intron, as well as 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#___An 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
- TxDb.Celegans.UCSC.ce6.ensGene - for C.elegans

orgAnn   organism annotation mapping such as org.Hs.egSYMBOL. Which lives in the org.Hs.eg.db package for humans.

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 takes on values from 1 to gRNA.size, representing all positions in the guide RNA (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. The default is 20 |
| canonical.PAM | Canonical PAM. The default is 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
- 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
- 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 extended-Sequence

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 = "GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU
    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", "CRISPRscan", "DeepCpf1"),
    mismatch.activity.file = system.file("extdata",
      "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 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

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)
```

---

deepCpf1                      *DeepCpf1 Algorithm for predicting CRISPR-Cpf1 gRNA Efficacy*

---

### Description

DeepCpf1 algorithm from https://doi.org/10.1038/nbt.4061, which takes in 34 bp target sequences with/without chromatin accessibility information and returns predicted CRISPR-Cpf1 gRNA efficacy for each input sequence.

### Usage

```
deepCpf1(extendedSequence, chrom_acc)
```

### Arguments

extendedSequence

Sequences containing gRNA plus PAM plus flanking sequences. Each sequence should be 34 bp long as specified by http://deepcrispr.info/, i.e., 4bp before the 5' PAM, 4bp PAM, 20bp gRNA, and 6bp after 3' of gRNA.

chrom_acc      Optional binary variable indicating chromatin accessibility information with 1 indicating accessible and 0 not accessible.

### Details

Having chromatin accessibility information will aid in the accuracy of the scores, but one can still get accurate scoring with only the 34 bp target sequences.

### Value

a numeric vector with prediced CRISPR-Cpf1 gRNA efficacy taking into account chromatin accessibility information if accessibility information is provided

### Author(s)

Paul Scemama and Lihua Julie Zhu

**References**

Kim et al., Deep learning improves prediction of CRISPR–Cpf1 guide RNA activityNat Biotechnol 36, 239–241 (2018). https://doi.org/10.1038/nbt.4061

**Examples**

```
library(keras)
library(mltools)
library(dplyr)
library(data.table)

use_implementation("tensorflow")

extendedSequence <- c('GTTATTTGAGCAATGCCACTTAATAAACATGTAA',
 'TGACTTTGAATGGAGTCGTGAGCGCAAGAACGCT',
 'GTTATTTGAGCAATGCCACTTAATAAACATGTAA',
 'TGACTTTGAATGGAGTCGTGAGCGCAAGAACGCT')
chrom_acc <- c(0,1, 0, 1)

if (interactive()) {
 deepCpf1(extendedSequence = extendedSequence, chrom_acc = chrom_acc)
}
```

---

 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,
  gRNA.size = 20,
  PAM.location = "3prime",
  PAM.size = 3,
  featureWeightMatrixFile = system.file("extdata", "DoenchNBT2014.csv", package =
    "CRISPRseek"),
  rule.set = c("Root_RuleSet1_2014", "Root_RuleSet2_2016", "CRISPRscan", "DeepCpf1"),
  chrom_acc,
  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 offtarget
- chromEnd - end position of the offtarget
- 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#___An 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.en 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
- 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 |
| gRNA.size | The size of the gRNA, default 20 for spCas9 |
| 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 | PAM length, default 3 for spCas9 |
| 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. |
| chrom_acc | Optional binary variable indicating chromatin accessibility information with 1 indicating accessible and 0 not accessible. |

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", "DeepCpf1"),
  chrom_acc
)
```

## 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. For Cpf1,you may set it to 19 and 23. |
| 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.

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.

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

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.  Please note
                   that if specifying DeepCpf1, please specify other parameters accordingly for
                   CRISPR-Cpf1 gRNAs.

chrom_acc          Optional binary variable indicating chromatin accessibility information with 1
                   indicating accessible and 0 not accessible.

## 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"
     )
   if (interactive()) {
     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 = 26,
         calculategRNAEfficacy= TRUE,
         rule.set = "DeepCpf1",
        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 = 26,
             calculategRNAEfficacy= TRUE,
             rule.set = "DeepCpf1",
             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 = 26,
             calculategRNAEfficacy= TRUE,
             rule.set = "DeepCpf1",
             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

- IsMismatch.posX - Indicator variable indicating whether this position X is mismatch or not, (1 means yes and 0 means not). X takes on values from 1 to gRNA.size, representing all positions in the guide RNA (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 - 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

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 an 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 target-specific guide RNAs for CRISPR-Cas9 system in one function*

---

### Description

Design target-specific guide RNAs (gRNAs) and predict relative indel fequencies for CRISPR-Cas9 system by automatically calling findgRNAs, filtergRNAs, searchHits, buildFeatureVectorForScoring, 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 = "GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU
    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", "DeepCpf1"),
    chrom_acc,
    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. For Cpf1, you can set it to 19 and 23. |
| 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. |
| 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 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
- 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_
"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 identifi-
cation.

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 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#___An 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
- 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

chrom_acc     Optional binary variable indicating chromatin accessibility information with 1 indicating accessible and 0 not accessible.

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 frequencies.

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 editing 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 information of off targets

Summary.xls     - summary of the gRNAs

REcutDetails.xls

    - restriction enzyme cut sites of each gRNA

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

Kim et al., Deep learning improves prediction of CRISPR–Cpf1 guide RNA activityNat Biotechnol 36, 239–241 (2018). https://doi.org/10.1038/nbt.4061

**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.

       #### 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, e.g., Cpf1
       results <- offTargetAnalysis(inputFilePath = system.file("extdata",
             "cpf1-2.fa", package = "CRISPRseek"), findgRNAsWithREcutOnly =  FALSE,
          findPairedgRNAOnly = FALSE,
          annotatePaired = FALSE,
          BSgenomeName = Hsapiens,
          chromToSearch = "chr8",
          txdb = TxDb.Hsapiens.UCSC.hg19.knownGene,
          orgAnn = org.Hs.egSYMBOL, max.mismatch = 4,
          baseBeforegRNA = 8, baseAfterPAM = 26,
          rule.set = "DeepCpf1",
          overlap.gRNA.positions = c(19, 23),
          useEfficacyFromInputSeq = FALSE,
          outputDir = getwd(),
          overwrite = TRUE, PAM.location = "5prime",PAM.size = 4,
          PAM = "TTTN", PAM.pattern = "^TNNN", 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 = "GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU
    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", "DeepCpf1"),
    chrom_acc,
    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

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.

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 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
- 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 "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 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 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.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#___An 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
> - 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

chrom_acc           Optional binary variable indicating chromatin accessibility information with 1 indicating accessible and 0 not accessible.

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 required when useBSgenome is set to FALSE.

predIndelFreq       Default to FALSE. Set it to TRUE to output the predicted indels and their frequencies.

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 editing 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 information of off targets

Summary.xls        - summary of the gRNAs

REcutDetails.xls

- restriction enzyme cut sites of each gRNA

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.

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 Predict insertions and deletions induced by CRISPR/Cas9 editing

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

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

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

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")



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 takes on values from 1 to gRNA.size, representing all positions in the guide RNA (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
- 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 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)
hits <- searchHits(all.gRNAs[1],
    seqs = DNAString(
        "TAATATTTTAAAATCGGTGACGTGGGCCCAAAACGAGTGCAGTTCCAAAGGCACCCACCTGTGGCAG"),
    seqname = "myseq", max.mismatch = 10, outfile = "test_searchHits")
colnames(hits)
all.gRNAs <- findgRNAs(inputFilePath =
        DNAStringSet(
            "TAATATTTTAAAATCGGTGACGTGGGCCCAAAACGAGTGCAGTTCCAAAGGCACCCACCTGTGGCAG"),
```

```
                pairOutputFile = "pairedgRNAs.xls",
                findPairedgRNAOnly = FALSE,
                PAM = "TTTN", PAM.location = "5prime")
        hits <- searchHits(all.gRNAs[1], seqs = DNAString(
            "TAATATTTTAAAATCGGTGACGTGGGCCCAAAACGAGTGCAGTTCCAAAGGCACCCACCTGTGGCAG"),
            seqname = "myseq",
            max.mismatch = 0,
            outfile = "test_searchHits", PAM.location = "5prime",
            PAM.pattern = "^T[A|T]NN", allowed.mismatch.PAM = 0, PAM = "TTTN")
        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
- BSgenome.Drerio.UCSC.danRer7 - for Zv9

- BSgenome.Dmelanogaster.UCSC.dm3 - for dm3

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

chromToExclude      Specify the chromosome not to search, default to none, meaning to search chro-
                    mosomes 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,
                    "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 sp-
                    Cas9. 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 takes on values from 1 to gRNA.size, representing all
  positions in the guide RNA (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
- 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 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
- 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 after,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, seqs = x)
}
```

---

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 = 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,
  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
- 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)
```

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