Package ‘TAPseq’

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Type Package

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Version 1.14.1

Description Design primers for targeted single-cell RNA-seq used by TAP-seq. Create sequence templates for target gene panels and design gene-specific primers using Primer3. Potential off-targets can be estimated with BLAST. Requires working installations of Primer3 and BLASTn.

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BugReports https://github.com/argschwind/TAPseq/issues

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accessors

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Description

A set of functions for getting/setting/modifying the data stored in TsIO or TsIOList class objects.
accessors

Usage

sequence_id(x)

sequence_id(x) <- value

target_sequence(x)

target_sequence(x) <- value

beads_oligo(x)

beads_oligo(x) <- value

reverse_primer(x)

reverse_primer(x) <- value

target_annot(x)

target_annot(x) <- value

product_size_range(x)

product_size_range(x) <- value

primer_num_return(x)

primer_num_return(x) <- value

min_primer_region(x)

min_primer_region(x) <- value

primer_opt_tm(x)

primer_opt_tm(x) <- value

primer_min_tm(x)

primer_min_tm(x) <- value

primer_max_tm(x)

primer_max_tm(x) <- value

sequence_template(x)

tapseq_primers(x)
bone_marrow_genex

PCR products

Arguments

x A TsIO or TsIOList class object.
value A valid value to assign to the chosen slot.

Value

Returns the stored value(s) of a slot, or sets a new value.

Examples

# chr11 primers example data
data("chr11_primers")

data("chr11_primers")

# slot values of TsIO objects can be accessed using accessor functions
tsio <- chr11_primers[[1]]
sequence_id(tsio)
sequence_id(tsio) <- "Gene1"
sequence_id(tsio)

# some slots can only be obtained, but not set as filling these is part of the TAPseq workflow
tapseq_primers(tsio)
pcr_products(tsio)

# sequence templates can be created
sequence_template(tsio)

# values of TsIOList object slots can be extracted as well, but not set
tsio_list <- chr11_primers[1:2]
sequence_id(tsio_list)
target_sequence(tsio_list)
target_annot(tsio_list)
tapseq_primers(tsio_list)

Usage

bone_marrow_genex

Mouse bone marrow 10x data

Description

Subset of a 10x mouse bone marrow dataset taken from Baccin et al., 2019 (https://www.nature.com/articles/s41556-019-0439-6). Contains gene expression and cell type data for 362 cells.
**checkPrimers**

**Format**

object of Seurat class.

---

**checkPrimers**  
*Check primers for complementarity*

**Description**

Check a TAP-seq primer set, i.e. outer or inner primers for a target gene panel, for potential complementarity issues when multiplexing. Uses Primer3’s check_primer functionality.

**Usage**

```r
checkPrimers(
  object,
  primer_opt_tm = 63,
  primer_min_tm = 59,
  primer_max_tm = 66,
  thermo_params_path = NA,
  primer3_core = getOption("TAPseq.primer3_core")
)
```

```r
## S4 method for signature 'TsIO'
checkPrimers(
  object,
  primer_opt_tm = 63,
  primer_min_tm = 59,
  primer_max_tm = 66,
  thermo_params_path = NA,
  primer3_core = getOption("TAPseq.primer3_core")
)
```

```r
## S4 method for signature 'TsIOList'
checkPrimers(
  object,
  primer_opt_tm = 63,
  primer_min_tm = 59,
  primer_max_tm = 66,
  thermo_params_path = NA,
  primer3_core = getOption("TAPseq.primer3_core")
)
```

**Arguments**

- **object**: A TsIO or TsIOList object containing designed primers.
checkPrimers

primer_opt_tm, primer_min_tm, primer_max_tm
Optimal, minimum and maximum primer melting temperature. Should be the same values that were used when designing the primers.

thermo_params_path
Optional path (character) to the primer3_config directory. Only required when using Primer3 < 2.5.0.

primer3_core
Path (character) to the primer3_core executable. Usually this is inferred when loading/attaching the package.

Value
A data.frame with check_primers results.

Methods (by class)
- checkPrimers(TsIO): Check primers from TsIO objects.
- checkPrimers(TsIOList): Check primers from TsIOList objects.

See Also

Examples
library(ggplot2)

# chr11 primers example data
data("chr11_primers")

# pick best primers based on predicted off-targets for subset of all primers
best_primers <- pickPrimers(chr11_primers, n = 1, by = "off_targets")

# check for complementarity
## Not run:
comp <- checkPrimers(best_primers)

# plot complementarity scores for every pair. the lines indicate complementarity scores of 47, # the default value applied by Primer3 to identify high complementarity primer pairs
ggplot(comp, aes(x = primer_pair_compl_any_th, y = primer_pair_compl_end_th)) +
  geom_hline(aes(yintercept = 47), colour = "darkgray", linetype = "dashed") +
  geom_vline(aes(xintercept = 47), colour = "darkgray", linetype = "dashed") +
  geom_point(alpha = 0.25) +
  theme_bw()

## End(Not run)
check_tool_installation

Check required tools

Description
Check if required software is installed and return paths to executables (if found).

Usage
check_tool_installation()

Value
A list containing paths to required tools, if found, else NA.

chr11_genes

Chromosome 11 genes

Description
GENCODE exon annotations of all protein-coding genes within a genomic region on human chromosome 11.

Usage
chr11_genes

Format
object of GRanges class.
chr11_polyA_sites | Chromosome 11 polyA sites

Description
Example polyadenylation sites for expressed protein-coding genes within human chromosome 11 genomic region. This dataset was created using `inferPolyASites` on available K562 Drop-seq data. In a real-world application these sites would have to be pruned manually before further use.

Usage
chr11_polyA_sites

Format
object of `GRanges` class.

chr11_primers | Chromosome 11 primers

Description
Example of a `TsIOList` object containing input and output for chromosome 11 genes primer design.

Usage
chr11_primers

Format
object of `TsIOList` class.

chr11_truncated_txs | Chromosome 11 truncated transcripts

Description
Annotations of target gene transcripts within human chromosome 11 region that were truncated at inferred polyA sites using `truncateTxsPolyA`.

Usage
chr11_truncated_txs

Format
object of `GRangesList` class.
**chr11_truncated_txs_seq**

_Chromosome 11 truncated transcript sequences_

**Description**

Sequences of truncated transcripts within human chromosome 11 region that were extracted using `getTxsSeq`.

**Usage**

`chr11_truncated_txs_seq`

**Format**

Object of `DNAStringSet` class.

---

**createIORecord**

_Create boulder IO record_

**Description**

Takes a `TsIO` or `TsIOList` object and converts it into a boulder IO record for Primer3. Essentially it converts it into a list of character vectors that each contain the tag and the value in the form: "TAG=VALUE". More on this format can be found in the Primer3 manual.

**Usage**

```r
createIORecord(object, thermo_params_path = NA)
```

**Arguments**

- **object**
  - TsIO or TsIOList object for which a Primer3 boulder IO record should be created.
- **thermo_params_path**
  - Optional path (character) to the `primer3_config` directory. Only required when using Primer3 < 2.5.0.
**designPrimers**

Details

This function is usually not needed by the user, because functions such as `designPrimers` handle IO record generation. However, this function can for instance be useful to generate IO records, write them to a file and pass them to Primer3 in the conventional way.

Value

A character vector containing the lines of the IO record.

Methods (by class)

- createIORecord(TsIO): Create IO record from TsIO objects.
- createIORecord(TsIOList): Create IO record from TsIO objects.

See Also


Examples

```r
# chromosome 11 truncated transcript sequences
data("chr11_truncated_txs_seq")

# create TsIOList object for primer desiging from sequence templates
obj <- TAPseqInput(chr11_truncated_txs_seq, product_size_range = c(350, 500))

# create boulder IO record
boulder_io <- createIORecord(obj)
head(boulder_io, 11)
```

---

**designPrimers**

*Design primers*

Description

Design primers based on TsIO or TsIOList objects. Creates boulder-IO records, passes input to Primer3 and parses the output.

Usage

```r
designPrimers(
  object,
  thermo_params_path = NA,
  primer3_core = getOption("TAPseq.primer3_core")
)
```

```r
## S4 method for signature 'TsIO'
designPrimers(
```

```r
```

```r
```
designPrimers

## S4 method for signature 'TsIOList'
designPrimers(
  object,
  thermo_params_path = NA,
  primer3_core = getOption("TAPseq.primer3_core")
)

Arguments

- **object**: TsIO or TsIOList object for which primers should be designed.
- **thermo_params_path**: Optional path (character) to the primer3_config directory. Only required when using Primer3 < 2.5.0.
- **primer3_core**: Path (character) to the primer3_core executable. Usually this is inferred when loading/attaching the package.

Value

A new TsIO or TsIOList object containing Primer3 output.

Methods (by class)

- `designPrimers(TsIO)`: Design primers using Primer3 from a TsIO object
- `designPrimers(TsIOList)`: Design primers using Primer3 from a TsIOList object

See Also

http://primer3.org/manual.html for Primer3 manual and TsIO for TsIO class objects.

Examples

```r
# chromosome 11 truncated transcript sequences and annotations
data("chr11_truncated_txs_seq")

# create TsIOList object for the first two sequence templates
tapseq_io <- TAPseqInput(chr11_truncated_txs_seq[1:2], product_size_range = c(350, 500))

# design primers
## Not run:
tapseq_io <- designPrimers(tapseq_io)
## End(Not run)

# designed primers are stored in the tapseq_primers slot
tapseq_primers(tapseq_io)
```
estimateOffTargets  Estimate primer off-targets using BLAST

Description

Functions to use BLAST to align TAP-seq primers against a genome and chromosome reference to estimate potential off-target binding sites.

Usage

createBLASTDb(
  genome,
  annot,
  blastdb,
  standard_chromosomes = TRUE,
  tx_id = "transcript_id",
  tx_name = "transcript_name",
  gene_name = "gene_name",
  gene_id = "gene_id",
  title = "TAP-seq_GT_DB",
  verbose = FALSE,
  makeblastdb = getOption("TAPseq.makeblastdb")
)

blastPrimers(
  object,
  blastdb,
  max_mismatch = 0,
  min_aligned = 0.75,
  primer_targets = c("transcript_id", "transcript_name", "gene_id", "gene_name"),
  tmpdir = tempdir(),
  blastn = getOption("TAPseq.blastn")
)

## S4 method for signature 'TsIO'
blastPrimers(
  object,
  blastdb,
  max_mismatch = 0,
  min_aligned = 0.75,
  primer_targets = c("transcript_id", "transcript_name", "gene_id", "gene_name"),
  tmpdir = tempdir(),
  blastn = getOption("TAPseq.blastn")
)

## S4 method for signature 'TsIOList'
blastPrimers(
estimateOffTargets

object,
blastdb,
max_mismatch = 0,
min_aligned = 0.75,
primer_targets = c("transcript_id", "transcript_name", "gene_id", "gene_name"),
tmpdir = tempdir(),
blastn = getOption("TAPseq.blastn")
)

Arguments

genome A BSgenome (or DNASTingSet) object containing the sequences of all chromosomes to obtain genome and transcript sequences.

annot A GRanges object containing all exons of transcripts to be considered.

blastdb TAP-seq BLAST database created with createBLASTDb.

standard_chromosomes (logical) Specifies whether only standard chromosomes should be included in output genome sequences (e.g. chr1-22, chrX, chrY, chrM for homo sapiens).

tx_id, tx_name, gene_name, gene_id (character) Column names in annot metadata containing transcript id, transcript name, gene name and gene id information.

title Optional title for BLAST database.

verbose (logical) If TRUE, additional information from makeblastdb is printed to the console. Default: FALSE.

makeblastdb Path to the makeblastdb executable. Usually this is inferred when loading/attaching the package.

object A TsIO or TsIOList object containing designed primers.

max_mismatch Maximum number of mismatches allowed for off-target hits (default: 0).

min_aligned Minimum portion of the primer sequence starting from the 3’ end that must align for off-target hits (default: 0.75).

primer_targets Specifies what should be used to identify primer targets for off-target identification. i.e. to what does the sequence_id in TsIO objects refer? Can be a subset of transcript_id, transcript_name, gene_id or gene_name. By default all 4 are checked. Set to NULL to disable any off-target identification. See Details for more information.

tmpdir Directory needed to store temporary files.

blastn Path (character) to the blastn executable. Usually this is inferred when loading/attaching the package.

Details

createBLASTDb creates a BLAST database containing genome and transcriptome sequences, which is required by blastPrimers. The created database contains both sequence files for BLAST and annotations to process the results.
Use `blastPrimers` to align designed TAP-seq primers against the created database to estimate off-target priming potential. Only hits where at least a specified portion of the sequence involving the 3’ end of the primer aligns with not more than a certain number of mismatches are considered.

`blastPrimers` counts the number of genes in which a primer has 1) exonic hits or 2) intronic hits, or 3) the number of hits in intergenic regions of the genome. The exonic and intronic counts should be interpreted as: "In how many genes does a primer have exonic (or intronic) hits?".

If a BLAST hit falls in both intronic and exonic regions of a given gene (i.e. exonic for one transcript, intronic for another transcript), only the exonic hit is counted for that gene. If a primer has for instance 3 BLAST hits in one gene, 2 exonic and 1 intronic, then one exonic hit and one intronic hit is counted for that gene.

If sequence IDs of the designed primers (`sequence_id`) refer to the target gene/transcripts and can be found in the BLAST database annotations via `primer_targets`, then only off-target hits are counted. This is usually the case if input for primer design was produced from target gene annotations.

**Value**

For `createBLASTDb` a directory containing the BLAST database. For `blastPrimers` a `TsIO` or `TsIOList` object with the number of potential off-targets added to the TAP-seq primer metadata.

**Functions**

- `createBLASTDb()`: Create a genome and transcriptome TAP-seq BLAST database
- `blastPrimers(TsIO)`: BLAST primers in a `TsIO` object
- `blastPrimers(TsIOList)`: BLAST primers in a `TsIOList` object

**Examples**

```r
## Not run:
library(BSgenome)

# human genome (hg38) BSgenome object
hg38 <- getBSgenome("BSgenome.Hsapiens.UCSC.hg38")

# get annotations for BLAST
annot_url <- paste0("ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release_32/", 
                   "gencode.v32.annotation.gtf.gz")
annot <- import(annot_url, format = "gtf")
blast_exons <- annot[annot$type == "exon" & annot$gene_type == "protein_coding"]

# build BLAST database
blastdb <- file.path(tempdir(), "blastdb")
createBLASTDb(genome = hg38, annot = blast_exons, blastdb = blastdb)

# chr11 primers example data (already contains off-targets, but we can overwrite them)
data("chr11_primers")
chr11_primers <- chr11_primers[1:3]  # only use a small subset for this example

# run blast to identify potential off-targets
```
exportPrimers

chr11_primers <- blastPrimers(chr11_primers, blastdb = blastdb)
tapseq_primers(chr11_primers)

# allow 1 mismatch between primer and off-target
chr11_primers <- blastPrimers(chr11_primers, blastdb = blastdb, max_mismatch = 1)
tapseq_primers(chr11_primers)

## End(Not run)

---

exportPrimers

Export TAP-seq primers

Description

A set of functions for TAP-seq primer export. Convert primers stored in TsIO or TsIOList objects to a simple data.frame for easier export. Or create BED format tracks for primers and write them to files for viewing in a genome browser (e.g. IGV).

Usage

createPrimerTrack(object, color = 1)

## S4 method for signature 'TsIO'
createPrimerTrack(object, color = 1)

## S4 method for signature 'TsIOList'
createPrimerTrack(object, color = 1)

exportPrimerTrack(..., con)

primerDataFrame(object)

## S4 method for signature 'TsIO'
primerDataFrame(object)

## S4 method for signature 'TsIOList'
primerDataFrame(object)

Arguments

- object: A TsIO or TsIOList object containing designed primers.
- color: Color used for the track (Default: black). Can be any of the three kinds of R color specifications.
- ...: One or more primer BED tracks created by createPrimerTrack.
- con: Connection to which tracks are written. Typically a .bed file.
exportPrimers

Value

For createPrimerTrack a data.frame with the primer track in BED format.

Functions

- createPrimerTrack(TsIO): Create primer BED track from TsIO objects
- createPrimerTrack(TsIOList): Create primer BED track from TsIOList objects
- exportPrimerTrack(): Export primer BED tracks files
- primerDataFrame(TsIO): Create a data.frame with primer data from TsIO
- primerDataFrame(TsIOList): Create a data.frame with primer data from TsIOList

Examples

```r
# chr11 primers example data
data("chr11_primers")

# pick best primers based on predicted off-targets
best_primers <- pickPrimers(chr11_primers, n = 1, by = "off_targets")

# primers data can be exported to a simple data.frame to e.g. write them to a .csv file
primers_df <- primerDataFrame(best_primers)
head(primers_df)

# primer binding sites in transcript sequences can be converted to genomic coordinates to create
# a BED track to visualize primers in a genome browser (e.g. IGV)

# create primer BED track with a fancy color
track <- createPrimerTrack(best_primers[1:5], color = "steelblue3")

# tracks can be written to .bed files using a little helper function (replace con = "" by a file)
exportPrimerTrack(track, con = "")

## Not run:
# one can easily export primer tracks for multiple TsIO or TsIOList objects (e.g. inner and
# outer nested primers) to one .bed file using different colors for each object. see vignette for
# a practical example:
vignette("taseq_primer_design", package = "TAPseq")

obj1 <- best_primers[1:5]
obj2 <- best_primers[6:10]
exportPrimerTrack(createPrimerTrack(obj1, color = "steelblue3"),
                  createPrimerTrack(obj2, color = "goldenrod1"),
                  con = "path/to/file.bed")

## End(Not run)
```
getTxsSeq

Get transcript sequences

Description

Extract the DNA sequences of all exons of transcript models and concatenate to one sequence per transcript. This is basically a wrapper for extractTranscriptSeqs, which makes sure that the exons are correctly sorted according to their position in the transcript (3’ to 5’).

Usage

getTxsSeq(transcripts, genome)

## S4 method for signature 'GRangesList'
getTxsSeq(transcripts, genome)

## S4 method for signature 'GRanges'
getTxsSeq(transcripts, genome)

Arguments

transcripts A GRanges or GRangesList object containing exons of transcripts for which sequences should be extracted. All exons in a GRanges object are assumed to belong to the same transcript. Multiple transcripts can be provided in a GRangesList object.

genome A BSgenome or DNAStringSet object containing chromosome sequences which should be used to extract transcript sequences. Although using a BSgenome object is the easiest way, the genome sequence could also be loaded from a FASTA file using readDNAStringSet.

Value

A DNAString or DNAStringSet object containing the transcript sequence(s).

Methods (by class)

- getTxsSeq(GRangesList): Obtain transcript sequence from GRangesList input
- getTxsSeq(GRanges): Obtain transcript sequence from GRanges input

Examples

library(BSgenome)

# protein-coding exons of transcripts within chr11 region
data("chr11_genes")
target_txs <- split(chr11_genes, f = chr11_genes$transcript_id)
get_gt_sequences

Get genome and transcriptome sequences

Description

Get DNA sequences of all chromosomes and all annotated transcripts of a genome. This function is used to create the sequences in createBLASTDb.

Usage

get_gt_sequences(
  genome,
  annot = NULL,
  tx_id = "transcript_id",
  tx_name = "transcript_name",
  gene_name = "gene_name",
  gene_id = "gene_id",
  include_genome = TRUE,
  standard_chromosomes = TRUE
)

Arguments

genome A BSgenome (or DNAStringSet) object containing the chromosome sequences to obtain genome and / or transcript sequences.
annot A GRanges object containing all exons of transcripts to be considered. If not specified, no transcript sequences will be included in the output fasta file.
tx_id, tx_name, gene_name, gene_id (character) Column names in annot metadata containing transcript id, transcript name, gene name and gene id information. These column are mandatory, but can contain internal names (e.g. "transcript-1" or "gene-1").
include_genome (logical) Specifies whether the genome sequence should be included in the output fasta file.
standard_chromosomes (logical) Specifies whether only standard chromosomes should be included in output genome sequences (e.g. chr1-22, chrX, chrY, chrM for homo sapiens).
compress (logical) Create a gzipped output fasta file.

Value

A DNAStringSet object containing the genome and transcriptome sequences.
inferPolyASites

**Description**

Infer polyA sites from 10X, Drop-seq or similar 3’ enriched sequencing data. Simple function that looks for peaks in read coverage to estimate potential polyA sites. Default parameters are chosen because they work reasonably well with the example data, but they should typically be empirically selected by verifying the output.

**Usage**

```r
inferPolyASites(
  genes,
  bam,
  polyA_downstream = 100,
  min_cvrg = 0,
  wdsize = 200,
  by = 1,
  extend_downstream = 0,
  perc_threshold = 0.9,
  parallel = FALSE
)
```

**Arguments**

- `genes` (GRangesList): Object containing annotations of genes for which polyA sites are to be estimated.
- `bam`: Path to .bam file containing aligned reads used for polyA site estimation.
- `polyA_downstream` (numeric): How far downstream of a peak in coverage are polyA sites expected? Somewhat depends on input DNA fragment size. (default: 100).
- `min_cvrg` (numeric): Minimal coverage for peaks to be considered for polyA site estimation (default: 0).
- `wdsize` (numeric): Window size to estimate sequencing coverage along transcripts (default: 200).
- `by` (numeric): Steps in basepairs in which the sliding window should be moved along transcripts to estimate smooth coverage (default: 1).
- `extend_downstream` (numeric): To which amount should transcript annotations be extended downstream when estimating polyA sites (default: 0). A reasonable value (e.g. 100-200 bp) allows to account for polyA sites that fall a few basepairs downstream of terminal exons.
perc_threshold  (numeric) Only sequencing coverage peaks within perc_threshold percentile of coverage are considered for polyA site estimation (default: 0.9). Avoids that small peaks that in coverage are considered, resulting in many false polyA sites.

parallel  (logical) Triggers parallel computing using the BioCParallel-package package. This requires that a parallel back-end was registered prior to executing the function. (default: FALSE).

Value

A GRanges object containing coordinates of estimated polyadenylation sites.

Examples

library(GenomicRanges)

# protein-coding exons of genes within chr11 region
data("chr11_genes")
target_genes <- split(chr11_genes, f = chr11_genes$gene_name)

# subset of target genes for quick example
target_genes <- target_genes[18:27]

# bam file containing aligned Drop-seq reads
dropseq_bam <- system.file("extdata", "chr11_k562_dropseq.bam", package = "TAPseq")

# infer polyA sites for all target genes with adjusted parameters. parameter values depend on the
# input data and at this stage it's best to try different settings and check the results
polyA_sites <- inferPolyASites(target_genes, bam = dropseq_bam, polyA_downstream = 50,
wdsize = 100, min_cvrg = 1, parallel = TRUE)

parsePrimer3Output  Parse Primer3 Output

Description

Parse Primer3 output and add to input TsIO or TsIOList object. This function is usually not used by the user, as designPrimers handles Primer3 output parsing.

Usage

parsePrimer3Output(object, primer3_output)

Arguments

object  The TsIO or TsIOList object used to design primers. No errors or warnings if this is a different TsIO or TsIOList object!

primer3_output  Character vector containing raw Primer3 output.
pickPrimers

Value

TsIO or TsIOList object with added Primer3 output

Examples

```r
## Not run:
# chromosome 11 truncated transcript sequences
data("chr11_truncated_txs_seq")

tapseq_io <- TAPseqInput(chr11_truncated_txs_seq[1:2], product_size_range = c(350, 500))

# create boulder IO records
io_record <- createIORecord(tapseq_io)

# design primers and store raw Primer3 output
primer3_core <- getOption("TAPseq.primer3_core")
primer3_output <- system2(command = primer3_core, input = io_record, stdout = TRUE)

# parse output and add it to input TsIO object(s)
tapseq_io <- parsePrimer3Output(tapseq_io, primer3_output)
tapseq_primers(tapseq_io)
## End(Not run)
```

pickPrimers

Pick best TAP-seq primers

Description

Pick based primers from designed primers for every target based on Primer3 penalty score or off-target priming estimated with blastPrimers.

Usage

```r
pickPrimers(object, n = 1, by = c("penalty", "off_targets"))
```

Arguments

- **object**: A TsIO or TsIOList object containing designed primers.
- **n**: The number of top primers to pick (default: 1, which returns the best primer).
- **by**: Attribute by which primers should be picked. Can be either penalty or off_targets.
selectTargetGenes

Details

If by is set to off_targets top primers are picked based on the lowest number of exonic, intronic and intergenic off-targets (in that priority).

Value

A TsIO or TsIOList object containing the picked primers.

Methods (by class)

- pickPrimers(TsIO): Pick best primers in a TsIO object
- pickPrimers(TsIOList): Pick best primers per target in a TsIOList object

Examples

```r
# chr11 primers examples
data("chr11_primers")

# pick the best primer per gene based on the fewest exonic, intronic and intergenic off-targets
# (in that order)
better_primers <- pickPrimers(chr11_primers, by = "off_targets")
tapseq_primers(better_primers)

# pick the best two primers per gene based on the lowest penalty score computed by Primer3
best_primers <- pickPrimers(chr11_primers, n = 2, by = "penalty")
tapseq_primers(best_primers)
```

Description

Select target genes that serve as markers for cell populations using a linear model with lasso regularization. How well a selected set of target genes discriminates between cell populations can be assessed in an intuitive way using UMAP visualization.

Usage

```r
selectTargetGenes(object, targets = NULL, expr_percentile = c(0.6, 0.99))
plotTargetGenes(object, target_genes, npcs = 15)
```
Arguments

- **object**: Seurat object containing single-cell RNA-seq data from which best marker genes for different cell populations should be learned. Needs to contain population identities for all cell.
- **targets**: Desired number of target genes. Approximately this many target genes will be returned. If set to NULL, the optimal number of target genes will be estimated using a cross-validation approach. Warning: The number of target genes might end up being very large!
- **expr_percentile**: Expression percentiles that candidate target genes need to fall into. Default is 60% to 99%, which excludes bottom 60% and top 1% expressed genes from markers.
- **target_genes**: (character) Target gene names.
- **npcs**: (integer) Number of principal components to use for UMAP.

Value

A character vector containing selected target gene identifiers.

Examples

```r
library(Seurat)

# example of mouse bone marrow 10x gene expression data
data("bone_marrow_genex")

# identify approximately 100 target genes that can be used to identify cell populations
target_genes <- selectTargetGenes(bone_marrow_genex, targets = 100)

# automatically identify the number of target genes to best identify cell populations using
# cross-validation. caution: this can lead to very large target gene panels!
target_genes_cv <- selectTargetGenes(bone_marrow_genex)

# create UMAP plots to compare cell type identification based on full dataset and selected 100
# target genes
plotTargetGenes(bone_marrow_genex, target_genes = target_genes)
```
Installation

In order to use the full functionality, Primer3 and BLAST need to be installed and added to PATH. Furthermore, the primer3_config directory containing important files for Primer3 should be located in the same directory as the primer3_core executable. If this is not practical, all functions interacting with Primer3 have arguments to specify the paths to these files.

For more information on installation see: https://github.com/argschwind/TAPseq.

TAPseqInput

Create TAPseq input from target sequences

Description

This function creates input for TAP-seq primer design from a DNAStringSet containing the target sequences (typically transcript sequences).

Usage

TAPseqInput(
  target_sequences,
  product_size_range,
  beads_oligo = NA,
  reverse_primer = "CTACACGACGCTCTTCCGATCT",
  target_annot = NULL,
  primer_num_return = 5,
  min_primer_region = 100,
  primer_opt_tm = 63,
  primer_min_tm = 59,
  primer_max_tm = 66
)

Arguments

  target_sequences
    A named DNAStringSet object containing all target sequences.

  product_size_range
    Numerical vector of length 2 specifying the desired length of the resulting amplicons.

  beads_oligo
    Beads-oligo-dT sequence for the used droplet sequencing protocol (10x, Dropseq). If nothing is specified (beads_oligo = NA), the 10x V3 Beads-oligo-dT sequence is used. Can be changed if primers are for instance designed for Dropseq. Any barcode bases need to be replaced by N.

  reverse_primer
    Reverse primer sequence used for all PCR reactions. Default is the 10x primer sequence: CTACACGACGCTCTTCGATCT.
target_annot  (optional) A named `GRangesList` object with transcript annotations in case the targets are transcripts of gene loci. If provided, each `GRanges` within the list should contain all exons of one targeted transcripts. Names need to be the same as for `target_sequences`.

primer_num_return  How many forward primers should be designed? (default: 5)

min_primer_region  Minimum sequence length required for primer design. Mostly relevant in case a sequence template is too short to allow the specified `product_size_range`.

primer_opt_tm, primer_min_tm, primer_max_tm  Optimal, minimum and maximum primer melting temperature. Set to NA to use Primer3s default values.

Value

`TsIOList` object.

Examples

# chromosome 11 truncated transcript sequences and annotations
data("chr11_truncated_txs_seq")

# create `TsIOList` object for primer design from target sequences
obj <- TAPseqInput(chr11_truncated_txs_seq, product_size_range = c(350, 500))

# transcript annotations can be added for optional genome browser tracks of designed primers
data("chr11_truncated_txs")
obj <- TAPseqInput(chr11_truncated_txs_seq, product_size_range = c(350, 500),
                   target_annot = chr11_truncated_txs)

# create input for primer design with Drop-seq instead of default 10x
ds_oligo <- "TTTTTTTAAGCAGTGGTATCAACGCAGAGTACNNNNNNNNNNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT"

ds_rev_primer <- "AAGCAGTGGTATCAACGCAGAGT"

ds_obj <- TAPseqInput(chr11_truncated_txs_seq, beads_oligo = ds_oligo,
                     reverse_primer = ds_rev_primer, product_size_range = c(350, 500),
                     primer_opt_tm = 62, primer_min_tm = 57, primer_max_tm = 65)

Description

Truncate transcripts at overlapping polyadenylation (polyA) sites to infer likely 3’ ends of transcripts. This is crucial to correctly design TAP-seq primers that amplify fragments of specific lengths. Typically the exons of all annotated transcripts per target gene are provided as input. If a polyA site overlaps a single transcript of a given gene, this transcript is truncated and returned. In case a polyA site overlaps multiple transcripts of the same gene, a “metatranscript” consisting of
all annotated exons of the overlapping transcripts is generated and truncated. No statements about
expressed transcripts can be made if no overlapping polyA sites are found for any transcripts of a
gene. In that case a "meta transcript" consisting of the merged exons of that gene is generated and
returned.

Usage

truncateTxsPolyA(
  transcripts,
  polyA_sites,
  extend_3prime_end = 0,
  polyA_select = c("downstream", "upstream", "score"),
  transcript_id = "transcript_id",
  gene_id = "gene_id",
  exon_number = "exon_number",
  ignore_strand = FALSE,
  parallel = FALSE
)

## S4 method for signature 'GRanges'
truncateTxsPolyA(
  transcripts,
  polyA_sites,
  extend_3prime_end = 0,
  polyA_select = c("downstream", "upstream", "score"),
  transcript_id = "transcript_id",
  gene_id = "gene_id",
  exon_number = "exon_number",
  ignore_strand = FALSE,
  parallel = FALSE
)

## S4 method for signature 'GRangesList'
truncateTxsPolyA(
  transcripts,
  polyA_sites,
  extend_3prime_end = 0,
  polyA_select = c("downstream", "upstream", "score"),
  transcript_id = "transcript_id",
  gene_id = "gene_id",
  exon_number = "exon_number",
  ignore_strand = FALSE,
  parallel = FALSE
)

Arguments

  transcripts  A GRanges or GRangesList object containing exons of the transcripts to be
              truncated. Transcripts for multiple genes can be provided as GRanges objects
**polyA_sites**  
A `GRanges` object containing the polyA sites. This needs to contain a metadata entry names "score" if the option `polyA_select = "score"` is used. PolyA sites can be either obtained via running `inferPolyASites` or imported from an existing .bed file (`BEDFile`).

**extend_3prime_end**  
Specifies how far (bp) 3’ ends of transcripts should be extended when looking for overlapping polyA sites (default = 0). This enables capturing of polyA sites that occur downstream of annotated 3’ ends.

**polyA_select**  
Specifies which heuristic should be used to select the polyA site used to truncate the transcripts if multiple overlapping polyA sites are found. By default "downstream" is used which chooses the most downstream polyA site. "score" selects the polyA site with the highest score, which corresponds to the read coverage when using `inferPolyASites` to estimate polyA sites.

**transcript_id** (character) Name of the column in the metadata of transcripts providing transcript id for each exon (default: "transcript_id"). Set to NULL to ignore transcript ids and assume that all exons per gene belong to the same transcript.

**gene_id, exon_number**  
(character) Optional names of columns in metadata of transcripts containing gene id and exon number. These are only used to create new metadata when merging multiple transcripts into a meta transcript.

**ignore_strand** (logical) Specifies whether the strand of polyA sites should be ignored when looking for overlapping polyA sites. Default is FALSE and therefore only polyA sites on the same strand as the transcripts are considered. PolyA sites with strand * has the same effect as `ignore_strand = TRUE`.  

**parallel** (logical) Triggers parallel computing using the BiocParallel package. This requires that a parallel back-end was registered prior to executing the function. (default: FALSE).

**Value**  
Either a `GRanges` or `GRangesList` object containing the truncated transcripts.

**Methods (by class)**  
- `truncateTxsPolyA(GRanges)`: Truncate transcripts of one gene provided as GRanges object  
- `truncateTxsPolyA(GRangesList)`: Truncate transcripts of multiple genes provided as GRangesList

**Examples**

```r  
library(GenomicRanges)  

# protein-coding exons of genes within chr11 region  
data("chr11_genes")  
target_genes <- split(chr11_genes, f = chr11_genes$gene_name)  

# only retain first 2 target genes, because truncating transcripts is currently computationally  
# quite costly. try using BiocParallel for parallelization (see ?truncateTxsPolyA).```
target_genes <- target_genes[1:2]

# example polyA sites for these genes
data("chr11_polyA_sites")

# truncate target genes at most downstream polyA site (default)
truncated_txs <- truncateTxsPolyA(target_genes, polyA_sites = chr11_polyA_sites)

# change polyA selection to "score" (read coverage of polyA sites) and extend 3' end of target
# genes by 50 bp (see ?truncateTxsPolyA).
truncated_txs <- truncateTxsPolyA(target_genes, polyA_sites = chr11_polyA_sites,
polyA_select = "score", extend_3prime_end = 50)

---

**TsIO-class**

**TsIO class**

### Description

TsIO objects store TAP-seq Primer3 input and output.

### Usage

```r
TsIO(
  sequence_id,
  target_sequence,
  beads_oligo,
  reverse_primer,
  product_size_range,
  target_annot = NULL,
  primer_num_return = 5,
  min_primer_region = 100,
  primer_opt_tm = NA,
  primer_min_tm = NA,
  primer_max_tm = NA
)
```

```r
## S4 method for signature 'TsIO'
sequence_id(x)

## S4 replacement method for signature 'TsIO'
sequence_id(x) <- value

## S4 method for signature 'TsIO'
target_sequence(x)

## S4 replacement method for signature 'TsIO'
target_sequence(x) <- value
```
## S4 method for signature 'TsIO'
beads_oligo(x)

## S4 replacement method for signature 'TsIO'
beads_oligo(x) <- value

## S4 method for signature 'TsIO'
reverse_primer(x)

## S4 replacement method for signature 'TsIO'
reverse_primer(x) <- value

## S4 method for signature 'TsIO'
target_annot(x)

## S4 replacement method for signature 'TsIO'
target_annot(x) <- value

## S4 method for signature 'TsIO'
product_size_range(x)

## S4 replacement method for signature 'TsIO'
product_size_range(x) <- value

## S4 method for signature 'TsIO'
primer_num_return(x)

## S4 replacement method for signature 'TsIO'
primer_num_return(x) <- value

## S4 method for signature 'TsIO'
min_primer_region(x)

## S4 replacement method for signature 'TsIO'
min_primer_region(x) <- value

## S4 method for signature 'TsIO'
primer_opt_tm(x)

## S4 replacement method for signature 'TsIO'
primer_opt_tm(x) <- value

## S4 method for signature 'TsIO'
primer_min_tm(x)

## S4 replacement method for signature 'TsIO'
primer_min_tm(x) <- value
## S4 method for signature 'TsIO'
primer_max_tm(x)

## S4 replacement method for signature 'TsIO'
primer_max_tm(x) <- value

## S4 method for signature 'TsIO'
sequence_template(x)

## S4 method for signature 'TsIO'
tapseq_primers(x)

## S4 method for signature 'TsIO'
pcr_products(x)

**Arguments**

- **sequence_id**: Name (character) of the target sequence, e.g. the gene name. It's advised to use meaningful sequence ids to safely assign designed primers to their targets.

- **target_sequence**: A DNAString or character object containing the target sequence for which primers should be designed. Usually a transcript sequence.

- **beads_oligo**: Beads-oligo-dT sequence for the used droplet sequencing protocol (10x, Drop-seq).

- **reverse_primer**: Reverse primer sequence used for all PCR reactions.

- **product_size_range**: Numerical vector of length 2 specifying the desired length of the resulting amplicons.

- **target_annot**: (optional) A GRanges object with transcript annotation in case the target is a transcript of a gene locus. If provided, it should contain all exons of the targeted transcript.

- **primer_num_return**: How many forward primers should be designed? (default: 5)

- **min_primer_region**: Minimum sequence length required for primer design. Mostly relevant in case the sequence template is too short to allow the specified product_size_range.

- **primer_opt_tm, primer_min_tm, primer_max_tm**: Optimal, minimum and maximum primer melting temperature.

- **x**: A TsIO object.

- **value**: A valid value to assign to the chosen slot.

- **tapseq_primers**: Slot where designed TAP-seq primers are stored. Not set by user.

- **pcr_products**: Slot where PCR products of primers are stored. Not set by user.
Details
The TsIO class is based on the Boulder IO records used by Primer3 (Primer3 manual). These objects are used to store the sequence templates and parameters needed for TAP-seq primer design. Primers designed with Primer3 are also stored in the same TsIO objects.

Use TsIO() to construct a new TsIO object from scratch.

Value
A TsIO object.

Methods (by generic)

- `sequence_id(TsIO)`: Get sequence_id
- `sequence_id(TsIO) <- value`: Set sequence_id
- `target_sequence(TsIO)`: Get target_sequence
- `target_sequence(TsIO) <- value`: Set target_sequence
- `beads_oligo(TsIO)`: Get beads_oligo
- `beads_oligo(TsIO) <- value`: Set beads_oligo
- `reverse_primer(TsIO)`: Get reverse_primer
- `reverse_primer(TsIO) <- value`: Set reverse_primer
- `target_annot(TsIO)`: Get target_annot
- `target_annot(TsIO) <- value`: Set target_annot
- `product_size_range(TsIO)`: Get product_size_range
- `product_size_range(TsIO) <- value`: Set product_size_range
- `primer_num_return(TsIO)`: Get primer_num_return
- `primer_num_return(TsIO) <- value`: Set primer_num_return
- `min_primer_region(TsIO)`: Get min_primer_region
- `min_primer_region(TsIO) <- value`: Set min_primer_region
- `primer_opt_tm(TsIO)`: Get primer_opt_tm
- `primer_opt_tm(TsIO) <- value`: Set primer_opt_tm
- `primer_min_tm(TsIO)`: Get primer_min_tm
- `primer_min_tm(TsIO) <- value`: Set primer_min_tm
- `primer_max_tm(TsIO)`: Get primer_max_tm
- `primer_max_tm(TsIO) <- value`: Set primer_max_tm
- `sequence_template(TsIO)`: Create sequence_template
- `tapseq_primers(TsIO)`: Get tapseq_primers
- `pcr_products(TsIO)`: Get pcr_products

See Also
Examples

```r
# get example transcript sequence
data("chr11_truncated_txs_seq")
tx_seq <- chr11_truncated_txs_seq[[1]]
tx_id <- names(chr11_truncated_txs_seq)[1]

# 10x beads-oligo-dt sequence
beads_oligo <- "CTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT"

# reverse primer used in all PCR reactions
reverse_primer <- "CTACACGACGCTCTCCGATCT"

# create TsIO object
obj <- TsIO(sequence_id = tx_id, target_sequence = tx_seq, beads_oligo = beads_oligo,
            reverse_primer = reverse_primer, product_size_range = c(350, 500))

# slot values can be accessed using accessor functions
sequence_id(obj)
sequence_id(obj) <- "Gene1"
sequence_id(obj)

# the sequence template (target sequence + reverse complement of beads-oligo-dt) for primer
# design can be viewed as well
sequence_template(obj)
```

TsIOList-class

**TsIOList class**

TsIOList class is a container to store multiple TsIO objects. This enables storing of Primer3 input and output for multiple target genes.

Usage

TsIOList(...)

```r
## S4 method for signature 'TsIOList'
sequence_id(x)

## S4 method for signature 'TsIOList'
target_sequence(x)

## S4 method for signature 'TsIOList'
sequence_template(x)

## S4 method for signature 'TsIOList'
target_annot(x)
```
## S4 method for signature 'TsIOList'
tapseq_primers(x)

## S4 method for signature 'TsIOList'
pcr_products(x)

### Arguments

...  
  Multiple TsIO objects from which a TsIOList object should be created.

x  
  A TsIOList object.

### Value

A TsIOList object.

### Methods (by generic)

- sequence_id(TsIOList): Get sequence_id
- target_sequence(TsIOList): Get target_sequence
- sequence_template(TsIOList): Create sequence_template
- target_annot(TsIOList): Get target_annot
- tapseq_primers(TsIOList): Get tapseq_primers
- pcr_products(TsIOList): Get pcr_products

### See Also

TsIO

### Examples

```r
# get example transcript sequences
data("chr11_truncated_txs_seq")
txs_seqs <- chr11_truncated_txs_seq[1:2]
txs_ids <- names(txs_seqs)

# 10x beads-oligo-dt sequence
beads_oligo <- "CTACACGACGCTCTTCCGATCTNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT"

# reverse primer used in all PCR reactions
reverse_primer <- "CTACACGACGCTCTTCCGATCT"

# create TsIO objects
tsio1 <- TsIO(sequence_id = txs_ids[1], target_sequence = txs_seqs[1],
              beads_oligo = beads_oligo, reverse_primer = reverse_primer,
              product_size_range = c(350, 500))

tsio2 <- TsIO(sequence_id = txs_ids[2], target_sequence = txs_seqs[2],
              beads_oligo = beads_oligo, reverse_primer = reverse_primer,
```
product_size_range = c(350, 500))

# create TsIOList object
obj <- TsIOList(tsio1 = tsio1, tsio2 = tsio2)

# it's noteworthy to mention that when creating a TsIOList from a DNAStringSet of target
# sequences, it's easier to use TAPseqInput()
?TAPseqInput

# as with TsIO objects, some values can be accessed using accessor functions
sequence_template(obj)
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