Package 'openPrimeR'

April 7, 2026

Title Multiplex PCR Primer Design and Analysis

Version 1.33.0

Description An implementation of methods for designing, evaluating, and comparing primer sets for multiplex PCR. Primers are designed by solving a set cover problem such that the number of covered template sequences is maximized with the smallest possible set of primers. To guarantee that high-quality primers are generated, only primers fulfilling constraints on their physicochemical properties are selected. A Shiny app providing a user interface for the functionalities of this package is provided by the 'openPrimeRui' package.

Depends R (>= 4.0.0)

License GPL-2

Encoding UTF-8

RoxygenNote 7.3.2

Imports Biostrings (>= 2.38.4), pwalign, XML (>= 3.98-1.4), scales (>= 0.4.0), reshape2 (>= 1.4.1), seqinr (>= 3.3-3), IRanges (>= 2.4.8), GenomicRanges (>= 1.22.4), ggplot2 (>= 2.1.0), plyr (>= 1.8.4), dplyr (>= 0.5.0), stringdist (>= 0.9.4.1), stringr (>= 1.0.0), RColorBrewer (>= 1.1-2), DECIPHER (>= 1.16.1), lpSolveAPI (>= 5.5.2.0-17), digest (>= 0.6.9), Hmisc (>= 3.17-4), ape (>= 3.5), BiocGenerics (>= 0.16.1), S4Vectors (>= 0.8.11), foreach (>= 1.4.3), magrittr (>= 1.5), uniqtag (>= 1.0), openxlsx (>= 4.0.17), grid (>= 3.1.0), grDevices (>= 3.1.0), stats (>= 3.1.0), utils (>= 3.1.0), methods (>= 3.1.0)

Suggests testthat (>= 1.0.2), knitr (>= 1.13), rmarkdown (>= 1.0), devtools (>= 1.12.0), doParallel (>= 1.0.10), pander (>= 0.6.0), learnr (>= 0.9)

SystemRequirements MAFFT (>= 7.305), OligoArrayAux (>= 3.8), ViennaRNA (>= 2.4.1), MELTING (>= 5.1.1), Pandoc (>= 1.12.3)

biocViews Software, Technology, Coverage, MultipleComparison

VignetteBuilder knitr

Collate 'AnalysisStats.R' 'Comparison.R' 'Data.R' 'templates.R'
'primers.R' 'IO.R' 'IO_view.R' 'Input.R' 'Ippolito.R'
'Output.R' 'Plots.R' 'PrimerDesign.R' 'PrimerEvaluation.R'
'RefCoverage.R' 'Scoring.R' 'SettingsDoc.R' 'TemplatesDoc.R'
'Tiller.R' 'ambiguity.R' 'check_stop_codons.R'
'con_annealing_temperature.R' 'con_dimerization.R'
'con_gc_clamp.R' 'con_gc_ratio.R' 'con_melting_temperature.R'
'con_primer_coverage.R' 'con_primer_efficiency.R'
'con_primer_secondary_structures.R' 'con_repeats.R'
'con_runs.R' 'con_template_secondary_structures.R'
'constraints.R' 'constraints_eval.R' 'errors.R' 'filters.R'
'helper_functions.R' 'initialize_primers.R'
'initialize_primers_tree.R' 'openPrimeR.R' 'optimization_ILP.R'
'optimization_algo.R' 'optimization_global.R'
'optimization_greedy.R' 'plots_comparison.R' 'settings.R'
'plots_constraints.R' 'plots_coverage.R' 'plots_filtering.R'
'primer_significance.R' 'startApp.R' 'zzz.R'

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

git_branch devel

git_last_commit 9ac7cf4

git_last_commit_date 2025-10-29

Repository Bioconductor 3.23

Date/Publication 2026-04-06

Author Matthias Döring [aut, cre],
Nico Pfeifer [aut]

Maintainer Matthias Döring <matthias-doering@gmx.de>

Contents

`openPrimeR-package` *Multiplex PCR Primer Design and Analysis.*

Description

With openPrimeR you can evaluate existing primers or design novel primers for multiplex polymerase chain reaction that are optimized with respect to the coverage of template sequences and the physicochemical properties of the primers.

Details

For designing primers, you just need the function `design_primers` from **openPrimeR**. As a minimal input, this function requires:

A set of template sequences You an load a `Templates` object with `read_templates`.

Settings for primer design You can load a `DesignSettings` object from a (supplied) XML file with `read_settings`. The settings can be easily customized using the setters `constraints`, `constraintLimits`, `cvg_constraints`, `conOptions`, and `PCR`.

For evaluating existing primers you can load a FASTA or CSV file containing the primers and templates of of interest using `read_primers` and `read_templates`, respectively. After evaluating the properties of the primers using `check_constraints`, you can interpret the results with several functions. For example, you can analyze the coverage of the template sequences using `get_cvg_stats`, determine the deviation from the target constraints using `plot_constraint_deviation`, or create a comprehensive report on the analyzed primers using `create_report`. In order to compare several primer sets with each other, you can create a table of the properties of the primer sets using `get_comparison_table` or create a full report, again using `create_report`.

Package options

openPrimeR uses the following options:

`openPrimeR.constraint_order` The identifiers of constraints in the order they are applied during the filtering procedure. This order is maintained when loading a `DesignSettings` object.

`openPrimeR.relax_order` The identifiers of constraints in the order in which they shall be relaxed during the relaxation procedure when designing primers.

`openPrimeR.plot_abbrev` The maximal number of allowed characters for tick labels in plots.

`openPrimeR.plot_colors` A named vector providing the identifiers of RColorBrewer palettes. Each vector entry provides the plotting colors for a specific type of stratification (i.e. by run, constraint, or primer). The palettes should provide at least eight colors.

Author(s)

Maintainer: Matthias Döring <matthias-doering@gmx.de>

Authors:

- Nico Pfeifer <pfeifer@informatik.uni-tuebingen.de>

AbstractConstraintSettings-class

AbstractClass for Constraint Settings.

Description

The ConstraintSettings class encapsulates the constraints on the physicochemical properties of primers.

Value

An AbstractConstraintSettings object.

Slots

`status` Named boolean vector indicating which of the possible constraints are active (TRUE) and which are not (FALSE).

`settings` Named list containing the settings of the active constraints

---

add.coverage.constraints

Addition of Coverage Constraints.

Description

Adds coverage constraints to ILP instance.

Usage

```
add.coverage.constraints(lprec, covered.templates, template.coverage)
```

Arguments

`lprec`	An ILP instance.
`covered.templates`	Indices of covered template sequences.
`template.coverage`	List containing the indices of covering primers for each template.

Value

lprec with coverage constraints.

`add.dimerization.constraints`
Addition of Dimerization Constraints

Description

Updates ILP formulation with dimerization constraints.

Usage

```
add.dimerization.constraints(lprec, D.idx, indices)
```

Arguments

`lprec`	An ILP instance.
`D.idx`	Data frame giving the indices of dimerizing primer pairs.
`indices`	Row indices for setting the dimerization constraints in `lprec`.

Value

`lprec` with added dimerization constraints.

`add.uniform.leaders.to.seqs`
Add Uniform Binding Regions.

Description

Augments a template data frame with uniform binding regions.

Usage

```
add.uniform.leaders.to.seqs(lex.seq, leaders)
```

Arguments

`lex.seq`	Template data frame
`leaders`	Data frame with uniform binding regions.

Value

Template data frame with updated binding regions.

adjust.ORF.start *Adjust ORF position*

Description

Adjusts the reading frame according to the position at which we consider a subsequence.

Usage

```
adjust.ORF.start(ORFs, seq.start)
```

Arguments

ORFs	the reading frames (0,1,2).
seq.start	the position where a sequence is extracted .

Value

The adjusted reading frames for the given start positions.

align.seqs *Multiple Sequence Alignment*

Description

Computes a multiple sequence alignment using MAFFT.

Usage

```
align.seqs(seqs, names)
```

Arguments

seqs	The sequences to be aligned.
names	The identifiers of the sequences.

Value

An alignment object as created from seqinr's read.alignment method.

References

Katoh, Misawa, Kuma, Miyata 2002 (Nucleic Acids Res. 30:3059-3066) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform.

align.structures *Formatting of Dimerization Structures.*

Description

Formats the given dimerization structures nicely.

Usage

```
align.structures(structs)
```

Arguments

s1	strutcs A character vector, where a block of 4 elements contains: sequence 1 (with removed overlaps), part of sequence 1 overlappign with sequence 2, part of sequence 2 overapping with sequence 1, and sequence 2 (with removed overlaps).

Value

A list of two elements givin the conformation of the first and the second sequence, respectively.

AnalysisStats *Primer Analysis Statistics.*

Description

`get_cvg_ratio` Determines the ratio of template sequences that are covered by the evaluated input primers. The ratio is in the interval [0,1] where 0 indicates 0% coverage (no templates covered) and 1 indicates 100% coverage (all templates covered).

`get_cvg_stats` Retrieve statistics on covered templates, either for a single or multiple primer sets.

`get_cvg_stats_primer` Creates a table summarizing the coverage events of individual primers.

`get_comparison_table` Creates an overview of the properties of multiple primer sets by providing the inter-quartile range of primer properties in bracket notation.

Usage

```
get_cvg_ratio(
  primer.df,
  template.df,
  allowed.mismatches = NULL,
  cvg.definition = c("constrained", "basic"),
  mode.directionality = NULL,
  as.char = FALSE
)
```

```
get_comparison_table(templates, primers, sample.name = NULL)

get_cvg_stats_primer(
  primer.df,
  template.df,
  cvg.definition = c("constrained", "basic")
)

get_cvg_stats(
  primers,
  templates,
  for.viewing = FALSE,
  total.percentages = FALSE,
  allowed.mismatches = Inf,
  cvg.definition = c("constrained", "basic")
)
```

Arguments

primer.df	A Primers object containing the primers.
template.df	A Templates object containing the template sequences corresponding to primer.df.
allowed.mismatches	The number of allowed mismatches for determining the coverage of the templates. By default, all annotated coverage events are considered.
cvg.definition	If cvg.definition is set to "constrained", the statistics for the expected coverage (after applying the coverage constraints) are retrieved. If cvg.definition is set to "basic", the coverage is determined solely by string matching (i.e. without applying the coverage constraints). By default, cvg.definition is set to "constrained".
mode.directionality	If mode.directionality is provided, the coverage of templates is computed for a specific direction of primers. Either "fw" (forward coverage only), "rev" (reverse coverage only), or "both" for both directions. By default, mode.directionality is NULL such that the directionality of the primers is determined automatically.
as.char	Whether the coverage ratio should be outputted as a percentage-formatted character vector. By default, as.char is set to FALSE such that a numeric is returned.
templates	If primers is an object of class Primers, please provide an object of class Templates containing the template sequences targeted by primers. If primers is a list, templates should be a list of Template objects.
primers	To retrieve statistics for a single primer set, please provide an object of class Primers containing a set of evaluated primers. To retrieve statistics for multiple primer sets, pelase provide a list with evaluated Primers objects.
sample.name	Either a single identifier or a character vector of identifiers for every Templates object in templates. By default, sample.name is NULL such that the Run annotations in the provided Templates objects are used.

for.viewing Whether the table should be formatted to be human-readable. By default, for.viewing is FALSE.

total.percentages
Whether group coverage percentages should be computed in relation to the total number of template sequences or in relation to the number of templates belonging to a specific group. By default, total.percentages is FALSE suc that the percentages are group-specific.

Details

The manner in which get_cvg_ratio determines the coverage ratio depends on the directionality of the input primers. If either only forward or reverse primers are inputted, the individual coverage of each primer is used to determine the overall coverage. If, however, forward and reverse primers are inputted at the same time, the coverage is defined by the intersection of binding events from both, forward and reverse primers.

For get_cvg_stats_primer, the cells corresponding to columns with numeric identifiers indicate the percentage of coverage events occurring with a certain number of mismatches. For example column 3 provides the number of coverage events where there are exactly three mismatches between primers and templates. The column Group_Coverage provides a listing of the percentage of covered templates per group.

Value

By default, get_cvg_ratio returns a numeric providing the expected primer coverage ratio. If as.char is TRUE, the output is provided as a percentage-formatted character vector. The attributes no_covered, no_templates, and covered_templates provide the number of covered templates, the total number of templates, and the IDs of covered templates, respectively.

get_comparison_table returns a data frame summarizing the properties of the provided primer data sets.

get_cvg_stats_primer returns a list with the following entries. cvg_per_nbr_mismatches contains a data frame listing the number of binding events broken down according to the number of expected mismatches between primers and templates. cvg_per_group contains a data frame listing the the coverage of individual primers per group of templates.

get_cvg_stats returns a data frame whose entries provide the coverage of templates per group of templates.

Examples

```
data(Ippolito)
# Determine the overall coverage
cvg.ratio <- get_cvg_ratio(primer.df, template.df)
# Determine the identitity coverage ratio
cvg.ratio.0 <- get_cvg_ratio(primer.df, template.df, allowed.mismatches = 0)

# Summarize the properties of multiple primer sets
data(Comparison)
tab <- get_comparison_table(template.data[1:3], primer.data[1:3], "IGH")

data(Ippolito)
```

```
# Determine coverage stats per primer
primer.cvg.stats <- get_cvg_stats_primer(primer.df, template.df)

# Coverage statistics for a single primer set
data(Ippolito)
cvg.stats <- get_cvg_stats(primer.df, template.df)
# Coverage statistics for multiple primer sets
data(Comparison)
cvg.stats.comp <- get_cvg_stats(primer.data[1:2], template.data[1:2])
```

`ancestor_of` *Tree Ancestry*

Description

Checks whether `ancestor.node` is an ancestor to the nodes specified in `test.node`.

Usage

```
ancestor_of(tree, ancestor.node, test.node)
```

Arguments

`tree`	The phylogenetic tree to be tested.
`ancestor.node`	A node to be checked for being an ancestor to `test.node`.
`test.node`	Possible descendants of `ancestor.node`.

Value

TRUE, if `ancestor.node` is an ancestor to any node in `test.node`.

`annealing.temp.rule.of.thumb`
Rule of thumb for annealing temperature

Description

Computes the annealing temperature using a rule of thumb

Usage

```
annealing.temp.rule.of.thumb(melting.temp)
```

Arguments

`melting.temp`	Melting temperatures of primers

Value

The annealing temperature corresponding to the input melting temperature.

`annotate.binding.events` *Annotation of Primer Binding Events.*

Description

Annotates whether primer binding events are in the allowed binding region or not.

Usage

```
annotate.binding.events(
  fw.binding,
  allowed.range,
  nbr.primers,
  allowed.region.definition = c("within", "any")
)
```

Arguments

`fw.binding`	IRanges with coverage information.
`allowed.range`	IRanges of the allowed binding ranges in the templates.
`nbr.primers`	Number of primers to consider.
`allowed.region.definition`	Definition of the allowed binding region

Value

IRanges with annotations of (preliminary) specificity and allowed binding. The field `all_binding` contains all binding regions, `on_target` contains all events in the target region, and `off_target` contains all off-target binding events.

`apply.constraint` *Application of Constraints*

Description

Checks whether the input values are within the specified limits.

Usage

```
apply.constraint(
  gc.ratio.fw,
  gc.ratio.rev,
  min.GC,
  max.GC,
  fw.idx,
  rev.idx,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

gc.ratio.fw	Forward values.
gc.ratio.rev	Reverse values.
min.GC	Minimal allowed value.
max.GC	Maximal allowed value.
fw.idx	Indices of forward values to consider.
rev.idx	Indices of reverse values to consider.
mode.directionality	Direction of primers

Value

Data frame with TRUE for values fulfilling the constraints, FALSE otherwise. Also returns FALSE if a data point is not available.

---

apply.constraint.list *Apply Constraints to a List.*

Description

Checks whether the input values are within the specified limits.

Usage

```
apply.constraint.list(
  gc.ratio.fw,
  gc.ratio.rev,
  min.GC,
  max.GC,
  fw.idx,
  rev.idx,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

gc.ratio.fw	Forward values (comma-separated strings).
gc.ratio.rev	Reverse values (comma-separated strings).
min.GC	Minimal allowed value.
max.GC	Maximal allowed value.
fw.idx	Indices of forward values to consider.
rev.idx	Indices of reverse values to consider.
mode.directionality	Direction of primers

Details

Applies a constraint to every element in a vector of comma separated strings. Applied when filtering covered seqs according to primer efficiency.

Value

Data frame with TRUE for values fulfilling the constraints, FALSE otherwise.

---

assign_binding_regions.character

Character Assignment of Binding Regions

Description

Generic method for assigning the binding region using individual binding regions.

Usage

```
## S3 method for class 'character'
assign_binding_regions(
  template.df,
  fw,
  rev,
  optimize.region = FALSE,
  primer.length = 20,
  gap.char = "-"
)
```

Arguments

template.df	Template data frame.
fw	FASTA file specifying the forward binding regions.
rev	FASTA file specifying the reverse binding regions.
optimize.region	Should the primer binding region be optimized using secondary structure prediction?
primer.length	Probe length for optimizing template secondary structure.
gap.char	The gap character for aligned sequences.

Value

Template data frame with assigned binding regions.

---

assign_binding_regions.numeric

Numeric Assignment of Binding Regions.

Description

Numeric S3 generic case for assigning binding regions.

Usage

```
## S3 method for class 'numeric'
assign_binding_regions(
  template.df,
  fw,
  rev,
  optimize.region = FALSE,
  primer.length = 20,
  gap.char = "-"
)
```

Arguments

template.df	Template data frame.
fw	Binding region data forward primers.
rev	Binding region data for reverse primers.
optimize.region	Should the primer binding region be optimized using secondary structure prediction?
primer.length	Probe length for optimizing template secondary structure.

Value

The template data frame with assigned binding regions.

augment.primer.cvg *Augmentation of Primer Coverage.*

Description

Computes the coverage for the primers in `primer.df` that is still missing such that the relaxation procedure can adjust appropriate constraints.

Usage

```
augment.primer.cvg(
  primer.df,
  template.df,
  settings,
  partial = FALSE,
  constraint = NULL
)
```

Arguments

`primer.df`	A `Primers` object for which the primer coverage shall be augmented.
`template.df`	A `Templates` object.
`settings`	A `DesignSettings` object giving the parameters for coverage computations.
`partial`	Whether all missing primer coverage values should be computed. If `partial` is `TRUE`, only the coverage values of the primers that were excluded due to the specified `constraint` are computed.
`constraint`	A character vector specifying the exclusion reason for which the partial augmentation should take place.

Value

A `Primers` object with augmented coverage entries.

batchify *Creates multiple Batches for Parallelization.*

Description

Creates multiple Batches for Parallelization.

Usage

```
batchify(tasks, annealing.temps = NULL)
```

Arguments

tasks	An integer vector with indices representing individual computations.
annealing.temps	Temperatures according to which to batchify.

Value

A list of lists containing indices corresponding to tasks, each list gives a batch.

---

batchify.simple *Simple Batchification*

Description

Simple Batchification

Usage

```
batchify.simple(tasks)
```

Arguments

tasks	The tasks to assign to individual batches.

Value

A list of lists containing indices corresponding to tasks, each list gives a batch.

---

batchify.temp *Batchification by Temperature.*

Description

Batchification by Temperature.

Usage

```
batchify.temp(tasks, annealing.temp)
```

Arguments

tasks	The tasks to assign to individual batches.
annealing.temp	The annealing temperatures according to which batches are to be created.

Value

A list of lists containing indices corresponding to tasks, each list gives a batch.

build.gain.df *Gain of Coverage by Excluded Primers.*

Description

Computes a data frame on the excluded sequences per constraint.

Usage

```
build.gain.df(candidate.df, constraint.settings, constraint.limits, relax.df)
```

Arguments

`candidate.df`	An object of class `Primers` containing excluded primers that are considered for addition to `filtered.df`.
`constraint.settings`	A list with the current constraint settings.
`constraint.limits`	The current constraint limits.
`relax.df`	Data frame with count of relaxations per constraint.

Value

A data frame with exclusion data.

build.ILP.df *Construction of ILP Results.*

Description

Constructs a data frame summarizing the properties of an ILP solution.

Usage

```
build.ILP.df(
  ILP,
  vars,
  primer.df,
  template.df,
  i,
  target.temp,
  time = NA,
  deltaG_Cutoff = NA,
  deltaG_Limit = NA
)
```

Arguments

ILP	A solved ILP instance.
vars	The ILP decision variables.
primer.df	The primer data frame correspdong to the ILP.
template.df	The template data frame.
i	Index for the ILP.
target.temp	Target melting temperature in Celsius.
time	Runtime of the ILP.
deltaG_Cutoff	Free energy cutoff used for the dimerization constraint.
deltaG_Limit	The free energy boundary for dimerization.

Value

Data frame summarizing the ILP solution.

---

build.tool.overview *Creation of an Overview of Third-Party Tools.*

Description

Creates a table of required third-party tools and their installation status.

Usage

```
build.tool.overview(AVAILABLE.TOOLS, for.shiny = FALSE)
```

Arguments

AVAILBLE.TOOLS	A vector whose names give the required tools and whose entries give their installation status as logicals.
If	for.shiny is TRUE, provide the URLs for the tool using HTML.

Value

A data frame with information on third-part tools.

`build_leader_df` *Building of Leader Data Frame.*

Description

Constructs the leader data frame.

Usage

```
build_leader_df(
  direction = c("fw", "rev"),
  leader,
  start,
  end,
  ali.start,
  ali.end
)
```

Arguments

`direction`	The primer direction for which we are annotating binding regions.
`leader`	The binding region sequence.
`start`	The start positions of the binding region.
`end`	The end positions of the binding region.
`ali.start`	The start positions of the binding region in the aligned input.
`ali.end`	The end positions of the binding region in the aligned input.

Value

A data frame with binding region information.

`call.melt` *Thermodynamic melting temperature computations.*

Description

Computes the melting temperature for the input primers.

Usage

```
call.melt(
  primers,
  complements,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc
)
```

Arguments

primers	Character vector of primer strings.
complements	Character vector with complement sequences corresponding to primers.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.

Value

Melting temperature data frame.

References

Le Novère N. (2001). MELTING, computing the melting temperature of nucleic acid duplex. Bioinformatics, 17: 1226-1227. Dumousseau M., Rodriguez N., Juty N., Le Novère N. (2012) MELTING, a flexible platform to predict the melting temperatures of nucleic acids. BMC Bioinformatics, 13: 101.

---

call.melt.single *Thermodynamic melting temperature computations.*

Description

Computes the melting temperature for the input primers.

Usage

```
call.melt.single(
  primers,
  complements,
  out.file,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  ID
)
```

Arguments

primers	List of primer strings.
complements	List with corresponding complements.
out.file	Path to the file where MELTING will write the results.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
ID	identifiers of the input primers

Value

Melting temperature data frame.

---

cascaded.filter *Filtering for the Optimization*

Description

Filter primers according to constraints and relax constraints if necessary.

Usage

```
cascaded.filter(
  primer.df,
  template.df,
  settings,
  mode.directionality = c("fw", "rev", "both"),
  required.cvg = 1,
```

```
  target.temps = NULL,
  updateProgress = NULL,
  results.loc = NULL
)
```

Arguments

primer.df	Primer data frame.
template.df	Template data frame.
settings	Settings object.
mode.directionality	Primer direction.
required.cvg	Required ratio of covered templates. If required.cvg is set to 0, the constraints are not relaxed.
target.temps	Target melting temperature of the primers in Celsius. This argument is only required if we try to match the melting temperatures of another primer set, e.g. when first optimizing forward and then optimizing reverse primers.
updateProgress	Progress callback function for shiny.
results.loc	Directory where the filtering results should be stored.

Details

Constraints are relaxed if the required.cvg could not be reached with the input constraints.

Value

The filtered primer data frame with respect to required.cvg.

---

cascaded.filter.quick *Cascaded Filter*

Description

Filter primers in a cascaded fashion.

Usage

```
cascaded.filter.quick(
  primer.df,
  template.df,
  settings,
  to.compute.constraints,
  mode.directionality = c("fw", "rev", "both"),
  active.constraints = NULL,
  no.structures = FALSE,
  updateProgress = NULL
)
```

Arguments

primer.df	Primer data frame.
template.df	Template data frame.
settings	Settings object.
to.compute.constraints	Names of constraints that still have to be computed.
mode.directionality	Primer direction.
active.constraints	The constraints that are to be used for filtering. If active.constraints is NULL, all filtering constraints are used.
no.structures	Whether dimerization structures shall be computed.
updateProgress	Progress callback function for shiny.

Details

At each constraint evaluation all primers that do not fulfill the current constraint are removed.

Constraints that are specified in to.compute.constraints are computed on the fly.

Value

The filtered primer data frame.

---

cbind.Primers *cbind for Primers class.*

Description

Ensures that the cbind result has the appropriate class.

Usage

```
## S3 method for class 'Primers'
cbind(...)
```

Arguments

...	Parameters for cbind function.

Value

Column binded Primers data frame.

Examples

```
data(Ippolito)
primer.df <- cbind(primer.df, primer.df)
```

cbind.Templates *cbind for Template class.*

Description

Ensures that the cbind result has the appropriate class.

Usage

```
## S3 method for class 'Templates'
cbind(...)
```

Arguments

...	Parameters for cbind function.

Value

Column binded Templates data frame.

Examples

```
data(Ippolito)
template.df <- cbind(template.df, seq_len(nrow(template.df)))
```

cbind2,Primers,ANY-method *S4 cbind for Primers.*

Description

S4 cbind function for Primers.

S4 rbind function for Primers.

Slices a Primers data frame.

Stores data in a column of a Primers data frame.

Usage

```
## S4 method for signature 'Primers,ANY'
cbind2(x, y, ...)

## S4 method for signature 'Primers,ANY'
rbind2(x, y, ...)

## S4 method for signature 'Primers,ANY'
```

```
x[i, j, ..., drop = TRUE]

## S4 replacement method for signature 'Primers'
x$name <- value
```

Arguments

x	The Primers data frame.
y	Another data frame.
...	Other arguments to the slice operator.
i	The row index.
j	The column index.
drop	Simplify data frame?
name	The name of the column.
value	The values of the column.

Value

Cbinded primer data frame.

Rbinded primer data frame.

Subset of primer data frame.

Primer data frame with replaced column.

Examples

```
data(Ippolito)
primer.df <- cbind2(primer.df, seq_len(nrow(primer.df)))
data(Ippolito)
primer.df <- primer.df[1:2,]
data(Ippolito)
primer.df$Forward[1] <- "ctagcgggaccg"
```

---

cbind2,Templates,ANY-method

S4 cbind for Templates.

Description

S4 cbind function for Templates data frame.

S4 rbind function for templates.

Slicing of Templates data frame object.

Set a column in a Templates data frame.

Usage

```
## S4 method for signature 'Templates,ANY'
cbind2(x, y, ...)

## S4 method for signature 'Templates,ANY'
rbind2(x, y, ...)

## S4 method for signature 'Templates,ANY'
x[i, j, ..., drop = TRUE]

## S4 replacement method for signature 'Templates'
x$name <- value
```

Arguments

x	The Template data frame.
y	Another data frame.
...	Other arguments to the slice operator.
i	The row index.
j	The column index.
drop	Simplify data frame?
name	The name of the column.
value	The values of the column.

Value

Cbinded template data frame.

Rbinded template data frame.

Subsetted template data frame.

Templates with replaced column.

Examples

```
data(Ippolito)
template.df <- cbind2(template.df, seq_len(nrow(template.df)))
data(Ippolito)
template.df <- rbind2(template.df, template.df)
data(Ippolito)
template.df <- template.df[1:2,]
data(Ippolito)
template.df$ID[1] <- "newID"
```

check.3prime.hexamers *3' Hexamer Check.*

Description

Check whether the 3' hexamer of a primer is fully complementary to the corresponding region in the template.

Usage

```
check.3prime.hexamers(
  template.df,
  primer.df,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

`template.df`	Template data frame.
`primer.df`	Primer data frame.
`mode.directionality`	Primer directionality.

Value

Returns `TRUE` if the 3' hexamer of a primer is fully complementary to the corresponding template region and `FALSE` otherwise.

check.3prime.mismatches *3' Mismatch Check.*

Description

Check for mismatches at primer 3' ends.

Usage

```
check.3prime.mismatches(
  template.df,
  primer.df,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

`template.df`	Template data frame.
`primer.df`	Primer data frame.
`mode.directionality`	Primer directionality.

Value

Returns the distance of mismatches from the 3' terminal end of primers.

`check.init.primer.length` *Primer Length Check.*

Description

Checks whether it is possible to construct primers of the desired length.

Usage

```
check.init.primer.length(
  template.df,
  allowed.region.definition = c("within", "any"),
  primer.lengths,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

`template.df`	Template data frame.
`allowed.region.definition`	Definition of allowed binding regions.
`primer.lengths`	The desired lengths of the priemrs.
`mode.directionality`	The primer directionality.

Value

TRUE, if primers of the desired length can be constructed,

check.init.primer.length.single *Primer Length Check.*

Description

Checks whether it is possible to construct primers of the desired length.

Usage

```
check.init.primer.length.single(
  allowed,
  allowed.region.definition = c("within", "any"),
  min.len
)
```

Arguments

allowed	String containing the allowed binding sequence.
allowed.region.definition	Definition of allowed binding regions.
min.len	Minimal desired primer lengths.

Value

TRUE if primers of the desired length can be constructed, FALSE otherwise.

check.mutations *Identification of Mismatch Mutations.*

Description

Identifies primers that induce mutations due to mismatch binding.

Usage

```
check.mutations(
  primer.seq,
  pos.start,
  pos.end,
  template.df,
  covered.seqs,
  ORF.data,
  mode.directionality = c("fw", "rev"),
  mutation.types = c("stop_codon", "substitution")
)
```

Arguments

primer.seq	Primer sequence string.
pos.start	Binding position of primer (start).
pos.end	Binding position of primer (end).
template.df	Template data frame.
covered.seqs	Identifiers of covered templates.
ORF.data	Reading frame information of templates.
mode.directionality	Directionality of primers.
mutation.types	Character vector of the mutation types to be checked for.

Details

Checks for one primer and all covered templates whether any templates are bound with mismatches such that a forbidden mutation is induced. A boolean vector indicating which binding events induce a forbidden mutation is returned.

Value

TRUE if the primer.seq induces a mutation that is forbidden according to the provided mutation.types.

---

check.template.constraints

Check Constraints on Templates

Description

Transforms the comma-separated input strings to a boolean representation.

Usage

```
check.template.constraints(template.constraints)
```

Arguments

template.constraints	Strings with comma-separated values to be turned to logical.

Value

List with boolean values

check.tool.function *Check Functionality of Third-Party Tools.*

Description

Checks whether all required tools should work.

Usage

```
check.tool.function(frontend = FALSE)
```

Arguments

frontend	Whether tool functionality shall be checked for the frontend.

Value

TRUE for each functioning tool, FALSE for non-functioning tools.

check.tool.installation *Check Tool Installation*

Description

Checks whether all required tools are installed.

Usage

```
check.tool.installation(frontend = FALSE)
```

Arguments

frontend	Whether tool installation shall be checked for the frontend. If TRUE, dependencies that are required only by the frontend are considered additionally.

Value

TRUE for each installed tool, FALSE otherwise.

check_constraints_comparison *Batch Procedure for Evaluating Primer Sets.*

Description

Batch Procedure for Evaluating Primer Sets.

Usage

```
check_constraints_comparison(
  primer.data,
  template.data,
  settings,
  active.constraints = names(constraints(settings)),
  to.compute.constraints = active.constraints,
  for.shiny = FALSE,
  updateProgress = NULL
)
```

Arguments

primer.data	A list of objects of class Primers.
template.data	A list of objects of class Templates corresponding to primer.data.
settings	An object of class DesignSettings.
active.constraints	A character vector providing identifiers of constraints to be considered.
to.compute.constraints	A character vector providing identifiers of constraints to be computed.
for.shiny	A logical indicating whether the results are indicated for the Shiny app or not.
updateProgress	A callback function to track progress in the Shiny app.

Value

A list with objects of class Primers.

Examples

```
## Not run:
data(Comparison)
eval.data <- check_constraints_comparison(primer.data[1:2], template.data[1:2], settings)

## End(Not run)
```

check_constraint_settings_validity *Check the Validity of the Constraint Settings.*

Description

Checks whether the status and the active constraints match. Determines whether the constraints are allowed/known.

Usage

```
check_constraint_settings_validity(object)
```

Arguments

object	An AbstractConstraintSettings object.

Value

TRUE if the constraint settings are valid, FALSE otherwise.

check_correspondence *Check of Primer and Template Correspondence.*

Description

Checks whether the primers relate to the correct templates.

Usage

```
check_correspondence(primer.df, template.df)
```

Arguments

primer.df	An object of class Primers.
template.df	An object of class Templates.

Value

TRUE if the primers and templates seem to correspond, FALSE otherwise.

check_cvg_constraints *Evaluation of Coverage Constraints.*

Description

Computes the biochemical properties specified in the `settings` object and determines whether the primers fulfill the required constraints.

Usage

```
check_cvg_constraints(
  primer.df,
  template.df,
  settings,
  active.constraints = names(cvg_constraints(settings)),
  to.compute.constraints = active.constraints,
  for.shiny = FALSE,
  updateProgress = NULL
)
```

Arguments

`primer.df`	A `Primers` object containing the primers to be checked.
`template.df`	A `Templates` object containing the template sequences corresponding to the primers.
`settings`	A `DesignSettings` object containing the coverage constraints to be checked and their settings.
`active.constraints`	Identifiers of constraints that are to be checked.
`to.compute.constraints`	Constraints that are to be computed.
`for.shiny`	Whether to format output for HTML.
`updateProgress`	Progress callback function for shiny.

Value

A `Primers` object with with columns for each constraint in `active.constraints`.

Note

Please note that some constraints can only be computed if additional software is installed, please see `DesignSettings` for an overview.

check_interval *Check Constraint Intervals*

Description

Checks the validity of constraint intervals.

Usage

```
check_interval(constraints)
```

Arguments

constraints	A list with constraint settings.

Value

TRUE, if all constraints specificy valid intervals, FALSE otherwise.

check_limits *Validity Check for Limits.*

Description

Checks whether the constraint limits are at least as general as the constraint settings. This ensures that the relaxation works in the proper direction.

Usage

```
check_limits(constraint.settings, constraint.limits)
```

Arguments

constraint.settings	A list with the constraint settings.
constraint.limits	A list with the constraint relaxation limits.

Value

TRUE if the limits are at least as wide as the constraints, FALSE otherwise.

check_limit_value *Check of limit correctness.*

Description

Checks whether a constraint limit is more general than the setting.

Usage

```
check_limit_value(setting, limit)
```

Arguments

setting	A single constraint setting.
limit	A single constraint limit.

Value

A vector containing TRUE if the limit is more general than the constraint setting and FALSE otherwise.

check_names *Check Setting Names.*

Description

Checks whether the specified settings hvae the correct names.

Usage

```
check_names(known.options, input.options)
```

Arguments

known.options	Allowed setting names.
input.options	Input setting names

Value

Mapping of input.options to known.options or NULL if invalid.

check_report_deps *Check for Report Dependencies.*

Description

Checks whether the dependencies for rmarkdown::render() are fulfilled.

Usage

```
check_report_deps()
```

Value

A logical vector giving the dependency availability status.

check_restriction_sites_single *Identification of Sequence Restriction Sites.*

Description

Checks the input sequences `seqs` for the presence of restriction sites. By removing the restriction sites from a primer set, it is possible to identify the coverage of the primers (e.g. using `check_constraints`) discounting for the impact of the mismatching bases caused by the insert.

Usage

```
check_restriction_sites_single(
  primer.seqs,
  template.seqs,
  adapter.action,
  direction = c("fw", "rev"),
  selected = NULL,
  only.confident.calls = TRUE,
  updateProgress = NULL
)
```

Arguments

`primer.seqs`	Nucleotide sequences of primers to be checked for restriction sites in terms of a `DNAStringSet` object.
`template.seqs`	A `DNAStringSet` object with nucleotide sequences containing the templates corresponding to `seqs`.

adapter.action	The action to be performed when adapter sequences are found. Either "warn" to issue warning about adapter sequences or "rm" to remove identified adapter sequences.
selected	Names of restriction sites that are to be checked. By default selected is NULL in which case all REBASE restriction sites are checked.
only.confident.calls	Only output confident calls of restriction sites.
updateProgress	A Shiny progress callback function.
The	primer direction that is checked.

Value

A data frame with restriction sites, if any could be found.

References

Roberts, R.J., Vincze, T., Posfai, J., Macelis, D. (2010) REBASE–a database for DNA restriction and modification: enzymes, genes and genomes. Nucl. Acids Res. 38: D234-D236. http://rebase.neb.com

---

check_setting *Check Setting Validity.*

Description

Checks whether the input settings are valid or not.

Usage

```
check_setting(known.options, options, mandatory.options = NULL)
```

Arguments

known.options	Vector with names and classes of allowed options.
options	Active options to be checked.
mandatory.options	Fields that have to be present.

Value

TRUE if the setting is valid, FALSE otherwise.

check_settings_validity

Validity Check for DesignSettings.

Description

Validates whether a DesignSettings object has the correct structure.

Usage

```
check_settings_validity(object)
```

Arguments

object	A DesignSettings object to be checked for validity.

Value

TRUE if object is valid, FALSE otherwise.

combine.binding.events

Combination of Binding Events.

Description

Appends all binding events.

Usage

```
combine.binding.events(my.binding.fw, my.binding.rev, fw.m, rev.m)
```

Arguments

my.binding.fw	Forward binding events of individual primers.
my.binding.rev	Reverse binding events of individual primers.
fw.m	Forward binding events of paired primers.
rev.m	Reverse binding events of paired primers.

Value

IRanges of all binding events.

combine.strings *Combination of OligoArrayAux Structure Sequences.*

Description

Combines the input strings.

Usage

```
combine.strings(s1, s2)
```

Arguments

s1	A character vector to be combined with s2.
s2	A character vector to be included into s1.

Value

A character vector.

comp *Sequence complement*

Description

Complements the input sequence (re-write of seqinr comp function for gap support)

Usage

```
comp(seq, forceToLower = TRUE, ambiguous = FALSE)
```

Arguments

seq	Input char vector.
forceToLower	if TRUE the input is transformed to lower case.
ambiguous	if TRUE ambiguous IUPAC nucleotides are complemented.

Value

The complement of seq.

compare.constraints *Constraint list comparison*

Description

Determines whether two list with constraints are identical.

Usage

```
compare.constraints(A, B)
```

Arguments

A	First constraint list.
B	Second constraint list.

Value

TRUE if the constraints are identical, FALSE else.

comparison.cvg *Comparison Coverage Stats.*

Description

Computes coverage stats for primer comparison.

Usage

```
comparison.cvg(primer.data, template.data)
```

Arguments

primer.data	List with primer data frames.
template.data	List with template data frames.

Value

Coverage statistics for comparing primers.

comparison.stats.raw *Computation of Raw Stats for Primer Comparison*

Description

Computes raw stats for primer comparison.

Usage

```
comparison.stats.raw(primer.data, template.data)
```

Arguments

primer.data	List with primer data frames.
template.data	List with template data frames.

Value

Raw statistics for primer comparison.

complement.sequence *Sequence complement*

Description

Computes the complement of the input sequence.

Usage

```
complement.sequence(seq)
```

Arguments

seq	The input sequence strings.

Value

The complements of the input sequences.

compute.all.cross.dimers *Cross dimerization*

Description

Compute worst-case DeltaG data frame with all possible primer cross-dimers.

Usage

```
compute.all.cross.dimers(
  primer.df,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  annealing.temp,
  results = NULL,
  check.idx = NULL,
  for.shiny = FALSE,
  no.structures = FALSE
)
```

Arguments

primer.df	Input primers data frame.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
annealing.temp	The PCR annealing temperature.
results	(optional) Cross dimer data frame (unfiltered)
check.idx	Indices of primers for checking cross-dimerization.
for.shiny	Whether the table is inteded for HTML display.
no.structures	Whether dimerization structures shall not be outputted.

Value

Worst-case cross dimers.

`compute.all.cross.dimers.frontend` *Cross Dimerization.*

Description

Computes all cross dimers in a user-formatted way.

Usage

```
compute.all.cross.dimers.frontend(
  primer.df,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  annealing.temp,
  for.shiny = FALSE,
  no.structures = FALSE
)
```

Arguments

`primer.df`	Input primer data frame.
`primer_conc`	Primer concentration.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris buffer concentration.
`annealing.temp`	The PCR annealing temperature.
`for.shiny`	Whether to format the table for HTML output.
`no.structures`	Whether to compute structures of dimers.

Value

A formatted data frame with cross-dimerization infos

`compute.all.cross.dimers.unfiltered` *Cross dimerization*

Description

Compute DeltaG data frame for possible primer cross-dimers.

Usage

```
compute.all.cross.dimers.unfiltered(
  primer.df,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  annealing.temp,
  check.idx = NULL,
  for.shiny = FALSE,
  no.structures = FALSE
)
```

Arguments

`primer.df`	Input primers data frame.
`primer_conc`	Primer concentration.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris buffer concentration.
`annealing.temp`	The PCR annealing temperature.
`check.idx`	Indices of primers for checking cross-dimerization.
`for.shiny`	Whether to format for HTML output.
`no.structures`	Whether dimer structures shall not be determined. If `TRUE`, structures are not computed resulting in faster runtimes.

Value

All cross dimers.

`compute.all.primer.subsets.ILP` *Computation of Primer Subsets*

Description

Computes all optimal primer subsets and stores their plots.

Usage

```
compute.all.primer.subsets.ILP(
  primer.df,
  template.df,
  k,
  groups,
  cur.results.loc,
  required.cvg = 1
)
```

Arguments

`primer.df`	Primer data frame.
`template.df`	Template data frame.
`k`	Subset size-increment.
`groups`	Identifiers of template groups in order to limit coverage to certain groups of template sequences.
`cur.results.loc`	Location for storing the results.
`required.cvg`	The required coverage ratio.

Value

Write-out of results.

`compute.all.self.dimers` *Self dimers*

Description

Computes all possible self dimers for the primers in the input data frame.

Usage

```
compute.all.self.dimers(
  primer.df,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  annealing.temp,
  for.shiny = FALSE,
  no.structures = FALSE
)
```

Arguments

primer.df	Input primer data frame.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
annealing.temp	The PCR annealing temperature.
for.shiny	Whether the output is to be formatted for HTML.
no.structures	Whether dimerization structures shall be outputted.

Value

Data frame with thermodynamic information on all self dimers.

---

compute.all.self.dimers.frontend

Self Dimerization.

Description

Computes all self dimers in a user-formatted way.

Usage

```
compute.all.self.dimers.frontend(
  primer.df,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
```

```
  k_salt_conc,
  tris_salt_conc,
  annealing.temp,
  for.shiny = FALSE,
  no.structures = FALSE
)
```

Arguments

primer.df	Input primer data frame.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
annealing.temp	The PCR annealing temperature.
for.shiny	Whether to format the table for HTML output.
no.structures	Whether dimerization structures shall be outputted.

Value

A formatted data frame with self-dimerization infos

---

compute.basic.details *Computation of Coverage Details*

Description

Determines binding properties of primers.

Usage

```
compute.basic.details(
  binding,
  mode = c("on_target", "off_target"),
  template.df,
  primers,
  mode.directionality = c("fw", "rev", "both"),
  allowed.mismatches,
  allowed.other.binding.ratio,
  allowed.region.definition = c("within", "any"),
  updateProgress = NULL
)
```

Arguments

binding	An IRanges object with primer binding information.
mode	Either on_target for on-target binding or off_target for off-target binding.
template.df	Template data frame.
primers	Primer data frame.
mode.directionality	Primer directionality.
allowed.mismatches	The number of allowed mismatches per binding event.
allowed.other.binding.ratio	Ratio of primers that are allowed to bind to non-allowed regions. If allowed.other.binding.ratio >0 primers are allowed to bind at any location within the templates. However, a warning is given if the ratio of primers binding to non-target regions exceeds the allowed.other.binding.ratio.
allowed.region.definition	Definition of the target binding sites used for evaluating the coverage. If allowed.region.definition is within, primers have to lie within the allowed binding region. If allowed.region.definition is any, primers have to overlap with the allowed binding region. The default is that primers have to bind within the target binding region.
updateProgress	Progress callback function for shiny.

Value

Primer data frame with information on the covered template sequences.

---

compute.constraints *Computation of Constraints.*

Description

Computes the specified constraints for the input primers.

Usage

```
compute.constraints(
  primer.df,
  mode.directionality = c("fw", "rev", "both"),
  template.df,
  settings,
 active.constraints = c("primer_coverage", "primer_length", "primer_specificity",
    "gc_clamp", "gc_ratio", "no_runs", "no_repeats", "self_dimerization",
    "cross_dimerization", "melting_temp_range", "melting_temp_diff",
   "secondary_structure", "primer_efficiency", "annealing_DeltaG", "stop_codon",
   "terminal_mismatch_pos", "substitution", "hexamer_coverage", "coverage_model",
    "off_primer_efficiency", "off_annealing_DeltaG", "off_coverage_model"),
```

```
  no.structures = FALSE,
  for.shiny = FALSE,
  updateProgress = NULL
)
```

Arguments

primer.df	Primer data frame.
mode.directionality	Primer directionality.
template.df	Template data frame.
settings	A DesignSettings object.
active.constraints	Strings giving the constraints that are to be computed.
no.structures	Whether dimer structures shall be computed.
for.shiny	Whether to format output for HTML.
updateProgress	Progress callback function for shiny.

Value

A data frame with columns for each constraint in active.constraints.

---

compute.covered.Ta *Annealing temperature*

Description

Computes the annealing temperature using all binding events.

Usage

```
compute.covered.Ta(
  primer.df,
  mode.directionality = c("fw", "rev", "both"),
  template.df,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  primer_conc
)
```

Arguments

primer.df	Primer data frame.
mode.directionality	Primer directionality.
template.df	Template data frame
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
primer_conc	Primer concentration.

Value

The recommended annealing temperature.

---

compute.dimer.matrix *Dimerization matrix*

Description

Computes a matrix indicating all dimerizing primers according to a DeltaG cutoff.

Usage

```
compute.dimer.matrix(G, deltaG.cutoff = -7)
```

Arguments

G	Matrix with free energies of all considered primer pairs.
deltaG.cutoff	Primers with free energies below the cutoff are considered dimerizing.

Value

Binary matrix with dimerization events according to the deltaG.cutoff. Contains '1' if primers (i,j) dimerize and '0' else.

`compute.efficiency` *Primer Efficiency.*

Description

Computes the efficiency of primer binding events for Taq polymerase.

Usage

```
compute.efficiency(
  fw.primers,
  fw.start,
  fw.end,
  covered,
  taqEfficiency,
  annealing.temp,
  primer_conc,
  sodium.eq.concentration,
  mode.directionality = c("fw", "rev"),
  seqs
)
```

Arguments

`fw.primers`	Primer sequence strings.
`fw.start`	Binding position (start).
`fw.end`	Binding position (end).
`covered`	List of covered template indices per primer.
`taqEfficiency`	Whether the efficiency shall be computed using a mismatch-model developed for Taq polymerases. The default setting is `TRUE`. Set `taqEfficiency` to `FALSE` if you are using another polymerase than Taq.
`annealing.temp`	Annealing temperature for which to evaluate efficiency.
`primer_conc`	Primer concentration.
`sodium.eq.concentration`	The sodium-equivalent concentration of ions.
`mode.directionality`	Primer directionality.
`seqs`	Template sequence strings.

Details

This function uses DECIPHER's CalculateEfficiencyPCR.

Value

The efficiencies of primer binding events.

References

Wright, Erik S., et al. "Exploiting extension bias in polymerase chain reaction to improve primer specificity in ensembles of nearly identical DNA templates." Environmental microbiology 16.5 (2014): 1354-1365.

`compute.empiric.melting.temp` *Non-Thermodynamic Computation of Melting Temperatures.*

Description

Computes the melting temperature of primers from an empiric formula.

Usage

```
compute.empiric.melting.temp(primer.df)
```

Arguments

`primer.df`	A `Primers` object.

Value

A data frame with melting temperature information for the primers.

`compute.gc.ratio` *GC ratio*

Description

Computes the ratio of G/Cs in a sequence.

Usage

```
compute.gc.ratio(x)
```

Arguments

`x`	Input sequence.

Details

In case of ambiguities, the mean GC ratio of all possible sequences is computed.

Value

The fraction of G/Cs in `x`.

compute.melting.temps *Computation of Melting Temperatures.*

Description

Use nearest-neighbor thermodynamic computations to find melting temperatures.

Usage

```
compute.melting.temps(
  primer.df,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

primer.df	Primer data frame.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
mode.directionality	Direction of primers

Value

Data frame with melting temperature info for the input primers.

compute.melting.temps.thermo *Computation of Thermodynamic Melting Temperatures.*

Description

Use nearest-neighbor thermodynamic computations to find melting temperatures.

Usage

```
compute.melting.temps.thermo(
  primer.df,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

`primer.df`	Primer data frame.
`primer_conc`	Primer concentration.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris buffer concentration.
`mode.directionality`	Direction of primers

Value

Data frame with melting temperature info for the input primers.

`compute.mismatch.table` *Mismatch overview table*

Description

Computes a table summarizing all of the mismatches caused by the primers in the input data frame.

Usage

```
compute.mismatch.table(
  primer.df,
  template.df,
  mode.directionality = c("fw", "rev")
)
```

Arguments

primer.df	Primer data frame.
template.df	Template data.
mode.directionality	Direction of primers.

Value

: A data frame summarizing all mismatches of the input primers with the input templates.

---

compute.primer.efficiencies *Primer Efficiency.*

Description

Computes the efficiency of primer binding events for Taq polymerase.

Usage

```
compute.primer.efficiencies(
  primer.df,
  template.df,
  annealing.temp,
  taqEfficiency,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  mode = c("on_target", "off_target")
)
```

Arguments

primer.df	Primer data frame.
template.df	Template data frame.
annealing.temp	Annealing temperature for which to evaluate efficiency.
taqEfficiency	Whether the efficiency shall be computed using a mismatch-model developed for Taq polymerases. The default setting is TRUE. Set taqEfficiency to FALSE if you are using another polymerase than Taq.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.

k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris ion concentration.
mode	Compute efficiencies for on-target coverage events (on_target) or off-target coverage events (off_target).

Details

This function uses DECIPHER's CalculateEfficiencyPCR.

Value

A list with the efficiency of every primer binding event.

Examples

```
data(Ippolito)
p <- PCR(settings)
# Requires OligoArrayAux software:
## Not run:
eff.df <- compute.primer.efficiencies(primer.df, template.df, 55,
             p$primer_concentration, p$Na_concentration,
             p$Mg_concentration, p$K_concentration, p$Tris_concentration)

## End(Not run)
```

---

compute.secondary.structures

Secondary Structure Computations.

Description

Computes the secondary structures of the input primers using ViennaRNA.

Usage

```
compute.secondary.structures(
  primer.df,
  mode.directionality = c("fw", "rev", "both"),
  annealing.temperature
)
```

Arguments

primer.df	Primer data frame.
mode.directionality	Direction of primers.
annealing.temperature	Temperatures at which to compute secondary structures for every primer

Value

Data frame with secondary structure information.

References

Lorenz, Ronny and Bernhart, Stephan H. and Höner zu Siederdissen, Christian and Tafer, Hakim and Flamm, Christoph and Stadler, Peter F. and Hofacker, Ivo L. ViennaRNA Package 2.0 Algorithms for Molecular Biology, 6:1 26, 2011, doi:10.1186/1748-7188-6-26

---

compute.sodium.equivalent.conc

Sodium-equivalent Concentration

Description

Computes the sodium-equivalent concentration for the input ion concentrations.

Usage

```
compute.sodium.equivalent.conc(
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc
)
```

Arguments

`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris buffer concentration.

Value

The sodium-equivalent concentration of the input ion concentrations.

References

Record, M. Thomas. "Effects of Na+ and Mg++ ions on the helix–coil transition of DNA." Biopolymers 14.10 (1975): 2137-2158.

Owczarzy, Richard, et al. "Predicting stability of DNA duplexes in solutions containing magnesium and monovalent cations." Biochemistry 47.19 (2008): 5336-5353.

Peyret, Nicolas. Prediction of nucleic acid hybridization: parameters and algorithms. Detroit: Wayne State University, 2000.

compute.structure.vienna *Computation of Secondary Structures with ViennaRNA.*

Description

Computes secondary structures using ViennaRNA.

Usage

```
compute.structure.vienna(
  seqs,
  annealing.temperature,
  folding.constraints = NULL,
  id = ""
)
```

Arguments

seqs	The input sequences for which structures shall be computed.
annealing.temperature	The temperature in degree Celsius at which to compute secondary structures.
folding.constraints	Character vector specifying the folding conditions for every input sequence. For example the constraint xxxxxx... would forbid folding in the first 6 bases and allow folding in the last 3 bases.
id	An identifier for storing the files
constraints	If provide

Value

A data frame with secondary structures.

compute.Ta *Annealing temperature*

Description

Computes the annealing temperature using all binding events.

Usage

```
compute.Ta(
  primer.df,
  template.df,
  mode.directionality = c("fw", "rev", "both"),
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  primer_conc
)
```

Arguments

`primer.df`	Primer data frame.
`template.df`	Template data frame
`mode.directionality`	Primer directionality.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris buffer concentration.
`primer_conc`	Primer concentration.

Value

All annealing temperatures for given binding events.

References

Rychlik, W. J. S. W., W. J. Spencer, and R. E. Rhoads. "Optimization of the annealing temperature for DNA amplification in vitro." Nucleic acids research 18.21 (1990): 6409-6412.

`compute.template.secondary.structures`

Template Secondary Structures

Description

Computes template secondary structures.

Usage

```
compute.template.secondary.structures(
  template.df,
  annealing.temperature,
  regions = NULL,
  constraints = NULL
)
```

Arguments

`template.df`	Template data frame.
`annealing.temperature`	Temperature [C] at which to compute secondary structures.
`regions`	List containing the positional intervals for which the template secondary structure should be computed.
`constraints`	String giving secondary structure constraints. For example `xxxxxx...` would forbid folding in the first 6 bases of a template with length 9 and allow folding in its last 3 bases.

Value

Data frame with info on template secondary structures.

`compute.Tm.baldino` *Baldino Formula*

Description

Computes the melting temperature using the formulation by Baldino.

Usage

```
compute.Tm.baldino(
  sequences,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  primer_conc
)
```

Arguments

sequences	Input sequence strings.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
primer_conc	Primer concentration.

Value

The melting temperature for the input sequences.

References

Rychlik, W. J. S. W., W. J. Spencer, and R. E. Rhoads. "Optimization of the annealing temperature for DNA amplification in vitro." Nucleic acids research 18.21 (1990): 6409-6412.

---

compute.Tm.sets *Cross-Dimerization Filtering*

Description

Removes cross-dimerizing primers from the input data.

Usage

```
compute.Tm.sets(
  primer.df,
  template.df,
  Tm.brackets,
  settings,
  mode.directionality = c("fw", "rev"),
  primer_conc,
  template_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  allowed.mismatches,
  allowed.other.binding.ratio,
  allowed.stop.codons,
  allowed.region.definition,
  disallowed.mismatch.pos,
  opti.mode = FALSE,
  required.cvg = NULL,
```

```
  primers.fw = NULL,
  diagnostic.location = NULL,
  updateProgress = NULL
)
```

Arguments

primer.df	Primer data frame.
template.df	Template data frame.
Tm.brackets	Data frame with target primer melting temperatures.
settings	A DesignSettings object.
mode.directionality	Identifier of strand for which primers shall be designed.
primer_conc	Primer concentration.
template_conc	Template concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris ion concentration.
allowed.mismatches	The number of mismatches primers are allowed to have with the templates.
allowed.other.binding.ratio	Ratio of primers allowed to bind to non-target regions.
allowed.stop.codons	Consider mismatch binding events that induce stop codons.
allowed.region.definition	Definition of the allowed region.
disallowed.mismatch.pos	The number of positions from the primer 3' end where mismatches should not be allowed. All primers binding templates with mismatches within disallowed.mismatch.pos from the 3' end are disregarded.
opti.mode	Compute optimization constraints and relax delta Tm if necessary.
required.cvg	Target coverage ratio.
primers.fw	Already designed primer sets for the target temperatures given in Tm.brackets. Used to determine cross-dimerization.
diagnostic.location	Directory for storing results.
updateProgress	Shiny progress callback function.
primers.rev	The primer data set to be filtered for cross-dimers.
opti.constraints	List with optimization constraint settings.
annealing.temp	The PCR annealing temperature.

Value

`primers.rev` with removed cross-dimerizing primers.

Primer data frames for every target temperature.

`compute.unique.covered.idx`

Unique Coverage Indices Computes the indices of templates that are covered uniquely covered by an individual primer.

Description

Unique Coverage Indices Computes the indices of templates that are covered uniquely covered by an individual primer.

Usage

```
compute.unique.covered.idx(primer.df, template.df)
```

Arguments

`primer.df`	Primer data frame.
`template.df`	Template data frame.

Value

Index of templates uniquely covered per input primer.

`compute_annealing_temp`

Annealing temperature.

Description

Identifies the optimal annealing temperature of a set of primers. If primers cover template sequences, the annealing temperature is computed using Rychlik's formula. Otherwise, the annealing temperature is determined using the rule of thumb based on the melting temperatures of the primers.

Usage

```
compute_annealing_temp(
  primer.df,
  mode.directionality,
  template.df,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  primer_conc
)
```

Arguments

primer.df	Primer data frame.
mode.directionality	Primer directionality.
template.df	Template data frame
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris buffer concentration.
primer_conc	Primer concentration.

Value

The optimal annealing temperature.

---

condition *Condition Constructor*

Description

Constructs a condition for custom errors.

Usage

```
condition(subclass, message, call = sys.call(-1), ...)
```

Arguments

subclass	String giving the specific error.
message	String giving the user message.
call	Environment object.
...	Other arguments for the output structure.

Value

A condition structure.

---

consecutive.GC.count *Consecutive GCs*

Description

Determines the maximum number of consecutive G/Cs

Usage

```
consecutive.GC.count(y, len)
```

Arguments

y	Positions where G/C occurs. Positions are numbered from 1 to 5 where 5 is the end of the primer.
len	Is the number of bases from the primer end considered.

Value

The maximal number of consecutive G/Cs.

---

constraints.to.df *Conversion of Constraints List to Data Frame.*

Description

Converts the input constraints to a data frame representation.

Usage

```
constraints.to.df(
  limit.constraints,
  out.names,
  format.type = c("backend", "shiny", "report")
)
```

Arguments

limit.constraints	A list with constraints.
out.names	The desired column names.
format.type	The type of formatting to be performed on the table

Value

A data frame giving an overview of the constraints.

---

constraints.xml.format *Constraint XML Format.*

Description

Format constraint settings for XML output.

Usage

```
constraints.xml.format(constraints, set.name)
```

Arguments

constraints	List with constraint settings.
set.name	Identifier for the constraint settings.

Value

XML string containing the constraint settings.

---

constraints_to_unit *Mapping of Constraints to Units.*

Description

Maps constraints to units for plotting.

Usage

```
constraints_to_unit(
  constraint,
  use.unit = TRUE,
  format.type = c("backend", "HTML", "report")
)
```

Arguments

constraint	The names of the constraints to convert to their plot identifiers (units).
use.unit	Whether constraint names should be annotated with their units.
use.HTML	Whether constraint units should be annotated with HTML units.

Value

A list of constraint names.

convert.from.iupac *Conversion from IUPAC nucleotides*

Description

Convert sequences with IUPAC ambiguity codes to all possible sequences without ambiguities.

Usage

```
convert.from.iupac(seqs)
```

Arguments

seqs	A vector of strings.

Value

A list containing the disambiguated input sequences.

convert.PCR.units *Conversion of PCR Units*

Description

Converts frontend PCR concentration units to the units used for the backend.

Usage

```
convert.PCR.units(pcr.settings, to.mol = TRUE)
```

Arguments

pcr.settings	List with several PCR settings (concentrations).
to.mol	If TRUE, convert to the molar concentration. If FALSE convert to the unit representation in the XML.

Value

List with concentrations for usage in the backend.

convert.temperature *Conversion between Celsius and Kelvin*

Description

Converts the input from Kelvin to Celsius or from Celsius to Kelvin.

Usage

```
convert.temperature(temp, temp.scale = c("K", "C"))
```

Arguments

temp	The input temperature.
temp.scale	The desired unit of the output temperature.

Details

If temp.scale is 'K', T_m is transformed from Celsius to Kelvin. If temp.scale is 'C', T_m is transformed from Kelvin to Celsius. The default is to transform from Celsius to Kelvin.

Value

Transforms the input temperature to the specified temp.scale.

convert.to.iupac *Merge sequences.*

Description

Merges the input sequences to one sequence containing IUPAC ambiguity codes.

Usage

```
convert.to.iupac(seqs)
```

Arguments

seqs	Vector of strings.

Value

Consensus sequence of seqs.

con_select *Quick Selection of Constraints.*

Description

Select constraints that can be used according to third-party tools quickly.

Usage

```
con_select(active.constraints)
```

Arguments

active.constraints	Identifiers of constraints.

Value

The identifiers of constraints that can be computed.

copy.melt.config *Copy MELTING Config File*

Description

Copies modified MELTING tandem mismatch file to the MELTING data folder.

Usage

```
copy.melt.config(melt.bin = NULL)
```

Value

TRUE if the file is available in the MELTING folder, FALSE otherwise.

`covered.primers.to.ID.string`
Conversion of Primer Indices to ID string

Description

Converts the input coverage indices to a comma-separated string with the template IDs.

Usage

```
covered.primers.to.ID.string(covered.primers, primer.df)
```

Arguments

`covered.primers`	Identifiers of primers covering sequences.
`primer.df`	Primer data frame.

Value

String containing the covered template IDs.

A string containing the IDs of covering primers.

`covered.seqs.to.ID.string`
Conversion of Template Coverage Indices to ID string

Description

Converts the input coverage indices to a comma-separated string with the template IDs.

Usage

```
covered.seqs.to.ID.string(covered.seqs, template.df)
```

Arguments

`covered.seqs`	Indices of covered template sequences.
`template.df`	Template data frame.

Value

A string containing the covered template IDs.

`covered.seqs.to.idx` *Conversion of Coverage Strings to Indices.*

Description

Converts the input coverage strings (comma separated template Identifiers) into indices.

Usage

```
covered.seqs.to.idx(covered.seqs, template.df)
```

Arguments

`covered.seqs`	Strings of covered sequences to be converted.
`template.df`	Template data frame containing the identifiers of templates

Value

Indices of covered templates.

`create.constraint.table` *Output a Constraint Overview Table*

Description

Outputs a table showing the values of constraints.

Usage

```
create.constraint.table(
  constraints,
  constraint.limits = NULL,
  constraints.used.fw = NULL,
  constraints.used.rev = NULL,
  format.type = c("backend", "shiny", "report")
)
```

Arguments

`constraints`	List with constraint settings.
`constraint.limits`	List with constraint limits.
`constraints.used.fw`	Constraints used for forward primer design.
`constraints.used.rev`	Constraints used for reverse primer design.
`format.type`	The type of formatting to be performed on entries.

Value

Data frame with summary of constraints.

`create.constraint.XML` *XML Output of Constraints*

Description

Creates an XML summarizing all settings.

Usage

```
create.constraint.XML(
  filtering.constraints,
  c.f.lim,
  cvg.constraints,
  PCR.settings,
  constraint.settings
)
```

Arguments

`filtering.constraints`	List with constraint settings for filtering.
`c.f.lim`	Relaxation limits for the filtering constraints.
`cvg.constraints`	List with constraints for coverage computations.
`PCR.settings`	Settings for the PCR.
`constraint.settings`	Other settings of constraints (e.g. coverage).

Value

String in XML format containing all constraint settings.

create.cvg.text *Coverage Info Text*

Description

Creates a string with information on the coverage

Usage

```
create.cvg.text(stats, selected.group = NULL, ident = NULL)
```

Arguments

stats	Data frame with coverage statistics.
selected.group	Retrieve information for a subgroup of templates only.
ident	An identifier for the coverage.

Value

A string with information on the coverage.

create.G.matrix *Create free energy matrix*

Description

Creates a matrix giving the deltaG values of all primers.

Usage

```
create.G.matrix(primer.df, G.df, primer.df.2 = NULL)
```

Arguments

primer.df	Primer data frame.
G.df	Free energy data for the primers.
primer.df.2	Optional second primer data frame

Value

Matrix with the smallest free dimerization energy for every primer pair.

`create.initial.primer.set` *Creation of an Initial Primer Set.*

Description

Creates an initial set of candidate primers for primer design.

Usage

```
create.initial.primer.set(
  template.df,
  primer.lengths,
  mode.directionality = c("fw", "rev"),
  sample,
  allowed.region.definition = c("within", "any"),
  init.algo = c("naive", "tree"),
  max.degen,
  conservation,
  updateProgress = NULL
)
```

Arguments

`template.df`	Template data frame.
`primer.lengths`	Vector containing the permissible primer lengths.
`mode.directionality`	Direction of primers to be created.
`sample`	Name of the template sample.
`allowed.region.definition`	Definition of the allowed binding region.
`init.algo`	Algorithm for initializing primers.
`max.degen`	Maximal allowed degeneration of created primers.
`conservation`	Required conservation of primers. The value of `conservation` should be in the range[0,1].
`updateProgress`	Shiny progress object.

Value

An initialized data frame of candidate primers.

create.k.mers *Creation of k-mers for multiple sequences.*

Description

Creation of k-mers for multiple sequences.

Usage

```
create.k.mers(seqs, k)
```

Arguments

k	The size of the k-mer.
seq	A character vector.

Value

A list with named character vectors, containing the k-mers.

create.kmer *Creation of k-mers of a single sequence.*

Description

Creation of k-mers of a single sequence.

Usage

```
create.kmer(seq, k)
```

Arguments

seq	A character vector.
k	The size of the k-mer.

Value

A names character vector, where the names are the relative positions of the k-mers and the values give the character vector of the k-mer.

create.options.table *Creation of a Table for Constraint Options.*

Description

Creation of a Table for Constraint Options.

Usage

```
create.options.table(
  other.settings,
  format.type = c("backend", "shiny", "report")
)
```

Arguments

other.settings	List with constraint options
format.type	How the table shall be formatted.

Value

A data frame.

create.other.table *Creation of a Table for Other Constraint Settings.*

Description

Creation of a Table for Other Constraint Settings.

Usage

```
create.other.table(other.settings, col.names, format.type)
```

Arguments

other.settings	List with other constraint settings.
format.type	How the table shall be formatted.

Value

A data frame.

create.PCR.table *Creation of a Table for PCR Conditions*

Description

Creation of a Table for PCR Conditions

Usage

```
create.PCR.table(other.settings, format.type = c("backend", "shiny", "report"))
```

Arguments

other.settings	List with PCR settings.
format.tyep	How the table shall be formatted.

Value

A data frame.

create.primer.ranges *Ranges for Initial Primers.*

Description

Creates a data frame indicating primer starts and ends.

Usage

```
create.primer.ranges(
  end.position,
  p.lens,
  start.position,
  step.size = 1,
  groups = NULL
)
```

Arguments

end.position	End positions of primers.
p.lens	Desired primer lengths.
step.size	A numeric giving the steps with which start positions are cycled. Should be 1 for primer design (evaluate all positions) and higher values can be used for windowing.
groups	Character vector with group annotation.
start.posiion	The start positions of primers.

Value

Data frame with ranges for initial primers.

`create.primers.naive` *Naive Initialization of Primers.*

Description

Initialize primers by extracting substrings from all templates.

Usage

```
create.primers.naive(
  seqs,
  seq.IDs,
  seq.groups,
  l.s,
  e.s,
  primer.lengths,
  allowed.region.definition,
  max.degen,
  sample = "",
  identifier = "",
  updateProgress = NULL
)
```

Arguments

`seqs`	The template sequence strings.
`seq.IDs`	The identifiers of the templates.
`seq.groups`	The group identifiers of the templates.
`l.s`	The positions where the allowed region starts for each template.
`e.s`	The positions where the allowed reigon ends for each template.
`primer.lengths`	Vector of desired primer lengths.
`allowed.region.definition`	Definition of the allowed region.
`max.degen`	Maximum allowed degeneracy of primers.
`sample`	Template sample identifier.
`updatProgress`	Shiny progress object.

Value

Data frame with initialized primer candidates.

`create.primers.tree` *Tree-based Initialization of Primers.*

Description

Creates a set of candidate primers using a tree-based algorithm.

Usage

```
create.primers.tree(
  seqs,
  seq.IDs,
  seq.groups,
  start,
  end,
  primer.lengths,
  allowed.region.definition,
  max.degen,
  conservation,
  sample = "",
  identifier = "",
  updateProgress = NULL
)
```

Arguments

`seqs`	Template sequences.
`seq.IDs`	Identifiers of template sequences.
`seq.groups`	Group identifiers of template sequences.
`start`	For each template the start of the interval where primers should be created.
`end`	For each template the end of the interval where primers should be created.
`primer.lengths`	Vector of desired primer lengths.
`allowed.region.definition`	Definition of allowed regions.
`max.degen`	Maximal degeneracy of primers.
`conservation`	Required conservation of template regions considered for the generation of primers. Conservation identifies the top conserved percentile of possible primers.
`sample`	Sample name for the analysis.
`identifier`	Identifier (e.g. for directionality).
`updateProgress`	Shiny progress object.

Details

First, primers are aligned and their sequence similarity is determined to compute a phylogenetic tree using hierarchical clustering. Next, the tree is traversed from leaves to top in order to identify groups of primers that can be merged (consensus) without exceeding the maximum degeneracy of primers.

Value

A vector with initialized primers.

---

create.Tm.brackets *Creation of Melting Temperature Groups*

Description

Creates a data frame identifying target melting temperatures of individual primer sets.

Usage

```
create.Tm.brackets(primers, template.df, settings, target.temps = NULL)
```

Arguments

primers	An object of class Primers for which to create groups based on melting temperatures.
template.df	An object of class Templates corresponding to the primers.
settings	A DesignSettings objects.
target.temps	Pre-defined target melting temperatures to use instead of automatically determining groups from the primers.

Value

Data frame with target melting temperatures for individual primer sets.

`create.uniform.leaders` *Uniform Binding Ranges.*

Description

Creates uniform binding regions for all templates.

Usage

```
create.uniform.leaders(fw.interval, rev.interval, template.df, gap.char)
```

Arguments

`fw.interval`	Binding region for forward templates.
`rev.interval`	Binding region for reverse templates.
`template.df`	Template data frame.
`gap.char`	The character for gaps in alignments.

Value

Data frame with binding region information.

`create_fulfilled_counts` *Creation of Fulfilled/Failed Constraint Counts.*

Description

Creates counts of fullfilled/failed constraints.

Usage

```
create_fulfilled_counts(primer.df, eval.cols = NULL)
```

Arguments

`primer.df`	An evaluated `Primers` object.
`eval.cols`	Evaluation columns in `primer.df` to consider. By default (`NULL`) all evaluation columns are considered.

Value

A data frame with the number of fulfilled/failed constraints for `primer.df`.

`create_report,list,list-method`
Creation of a PDF Report for Primer Comparison.

Description

Creates a PDF report for comparing multiple primers.

Usage

```
## S4 method for signature 'list,list'
create_report(
  primers,
  templates,
  fname,
  settings,
  sample.name = NULL,
  used.settings = NULL
)
```

Arguments

`primers`	A list with evaluated `Primers` objects.
`templates`	A list with `Templates` objects.
`fname`	A character vector giving the file to store the report in.
`settings`	A `DesignSettings` object.
`sample.name`	An identifier for your analysis.
`used.settings`	A named list (with fields "fw" and "rev") containing the forward/reverse used design settings.

Value

Creates a PDF file giving a report on the comparison of the input primers.

Note

Creating the report requires the external programs Pandoc (http://pandoc.org) and LateX (http://latex-project.org).

`create_report,Primers,Templates-method`
Creation of a PDF report.

Description

Creates a PDF report for a set of primers.

Usage

```
## S4 method for signature 'Primers,Templates'
create_report(
  primers,
  templates,
  fname,
  settings,
  sample.name,
  used.settings,
  required.cvg = 1
)
```

Arguments

`primers`	An evaluated `Primers` object.
`templates`	A `Templates` object.
`fname`	A character vector giving the file to store the report in.
`settings`	A `DesignSettings` object.
`sample.name`	An identifier for your analysis.
`used.settings`	A named list (with fields "fw" and "rev") containing the forward/reverse used design settings.
`required.cvg`	The required coverage ratio.

Value

Creates a PDF file reporting on the input primers.

Note

Creating the report requires the external programs Pandoc (http://pandoc.org) and LaTeX (http://latex-project.org).

Data *Data Sets.*

Description

`Ippolito` IGHV primer data from Ippolito et al.

`Tiller` IGHV primer data from Tiller et al.

`Comparison` Evaluated primer sets targeting the functional human IGH immunoglobulin genes. The sets were generated using the default evaluation settings of openPrimeR. The primer sets were gathered from IMGT and the literature.

`RefCoverage` Experimental results of multiplex PCR.

Usage

```
data(Comparison)

data(Ippolito)

data(RefCoverage)

data(Tiller)
```

Format

For the `RefCoverage` data set, the `feature.matrix` data frame contains the properties of the primer set from Tiller et al. as well as a primer set that was designed by openPrimeR. The column `Experimental_Coverage` indicates the experimentally determined coverage, while the other columns relate to properties of the primers that were computed with openPrimeR. The `ref.data` list contains the raw experimental coverage of individual primers from the primer sets from Tiller and openPrimeR, which both target templates from the IGH locus. The rows of the data frames indicate primers and the columns indicate IGH templates for which experimental coverage was determined. The cell entries are hex codes. Each hex code represents a color indicating a certain experimental coverage status. Hex codes representing red shades indicate no or little amplification, while hex codes for green shades indicate high yields.

For the `Ippolito` data set, `primer.df` provides a `Primers` object containing the evaluated set of primers from Tiller et al. `template.df` provides a `Templates` object containing functional, human IGHV templates for, and `settings` provides a `DesignSettings` object providing the used analysis settings.

For the `Comparison` data set, `primer.data` and `template.data` are lists of `Primers` and `Templates` objects, respectively.

For the `Tiller` data set, `tiller.primer.df` provides a `Primers` object, `tiller.template.df` provides the corresponding `Templates` object, and `tiller.settings` provides the `DesignSettings` object that was used for evaluating `tiller.primer.df`.

References

IMGT®, the international ImMunoGeneTics information system® http://www.imgt.org (founder and director: Marie-Paule Lefranc, Montpellier, France).

Ippolito GC, Hoi KH, Reddy ST, Carroll SM, Ge X, Rogosch T, Zemlin M, Shultz LD, Ellington AD, VanDenBerg CL, Georgiou G. 2012. Antibody Repertoires in Humanized NOD-scid-IL2R gamma null Mice and Human B Cells Reveals Human-Like Diversification and Tolerance Checkpoints in the Mouse. PLoS One 7:e35497.

Tiller, Thomas, et al. "Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning." Journal of immunological methods 329.1 (2008): 112-124.

Examples

```
# Load the comparison data
data(Comparison)
# Explore the first entry of the primer and template data:
primer.data[[1]]
template.data[[1]]
# Summarize the primer properties:
get_comparison_table(template.data, primer.data)

# Load the data from Ippolito et al.
data(Ippolito)
primer.df
template.df
constraints(settings)

# Load experimental PCR results
data(RefCoverage)

# Load the data from Tiller et al.
data(Tiller)
tiller.primer.df
tiller.template.df
constraints(tiller.settings)
```

---

design_primers.single *Design Primers for a Single Direction*

Description

Designs primers for a single direction.

Usage

```
design_primers.single(
  template.df,
  sample.name,
```

```
  mode.directionality = c("fw", "rev"),
  settings,
  timeout,
  opti.algo,
  allowed.region.definition,
  init.algo,
  max.degen,
  conservation,
  target.temps,
  required.cvg,
  fw.primers = NULL,
  cur.results.loc = NULL,
  primer.df = NULL,
  updateProgress = NULL
)
```

Arguments

`template.df`	Template data frame with sequences for which primers shall be designed.
`sample.name`	Identifier for the templates.
`mode.directionality`	Template strands for which primers shall be designed. Primers can be designed either only for forward strands, only for reverse strands, or both strand directions.
`settings`	A `DesignSettings` object specifying the criteria for designing primers.
`timeout`	Timeout in seconds for the optimization with ILPs.
`opti.algo`	The algorithm to be used for solving the primer set covering problem.
`allowed.region.definition`	Definition of the target binding sites used for evaluating the coverage. If `allowed.region.definition` is "within", primers have to lie within the allowed binding region. If `allowed.region.definition` is "any", primers have to overlap with the allowed binding region. The default is that primers have to bind within the target binding region.
`init.algo`	The algorithm to be used for initializing primers. If `init.algo` is `naive`, then primers are constructed from substrings of the input template sequences. If `init.algo` is `tree`, phylogenetic trees are used to form degenerate primers whose degeneracy is bounded by `max.degen`.
`max.degen`	Maximal degeneracy of merged primers.
`conservation`	When using the tree-based primer initialization, consider only the `conservation` percentile of regions with the highest conservation.
`target.temps`	Target melting temperatures for optimized primer sets in Celsius. Only required when optimizing primers for both strand directions and one optimization was already performed.
`required.cvg`	The target ratio of covered template sequences. If the target ratio cannot be reached, the constraint settings are relaxed up to the relaxation limits.

fw.primers	List with optimized primer data frames corresponding to target.temps. Only required for optimizing both strand directions and only in the second optimization run in order to check for cross dimerization.
cur.results.loc	Directory for storing results of the primer design procedure.
primer.df	A data frame of evaluated primer candidates that can be optimized directly.
updateProgress	Shiny progress callback function.

Value

A list containing the results of the primer design procedure:

opti: A Primers object representing the set of optimized primers.

all_results: A list containing the optimal results for each sampled melting temperature range in terms of a Primers object in case that the melting_temp_diff constraint was active. Otherwise, all_results only has a single entry representing a primer set relating to an undefined melting temperature.

used_constraints: A DesignSettings object with the (adjusted) analysis settings.

filtered: A Primers object containing the primer candidates that passed the filtering procedure and which gave rise to the final optimal set.

---

detect.gap.columns *Identification of Gappy Columns in Alignments.*

Description

Identification of Gappy Columns in Alignments.

Usage

```
detect.gap.columns(bins, gap.cutoff = 0.95, gap.char = "-")
```

Arguments

bins	A list of DNABin alignments.
gap.cutoff	The required percentage of gaps for consideration as a gap column.
gap.char	The gap character in the alignments.

Value

A list with indices giving the gap columns for every alignment in bins.

dimerization.table *Dimerization Table.*

Description

Summarizes how often individual primers dimerize according to the `deltaG.cutoff`.

Usage

```
dimerization.table(
  dimer.data,
  deltaG.cutoff,
  dimer.type = c("Self-Dimerization", "Cross-Dimerization")
)
```

Arguments

`dimer.data`	Data frame with dimerization data.
`deltaG.cutoff`	Free energy cutoff for dimerization.
`dimer.type`	String identifying whether `dimer.data` refers to cross-dimers or self-dimers?

Value

Data frame with dimer counts.

dir.copy *Copy Directories.*

Description

Copies a directory to another location.

Usage

```
dir.copy(src.dir, dest.dir, overwrite)
```

Arguments

`src.dir`	The directory to be copied.
`dest.dir`	The target directory.
`overwrite`	Overwrite existing files in `dest.dir`.

Value

TRUE if copying was successful, FALSE otherwise.

disambiguate.primers *Disambiguation of Primers.*

Description

Disambiguates ambiguous primer sequences into all possible sequences.

Usage

```
disambiguate.primers(p.df)
```

Arguments

p.df	Primer data frame.

Value

Data frame with disambiguated primers.

estimate.cvg *Estimation of Primer Coverage.*

Description

Estimates the possible coverage of primers using probes of size k and only considering perfect matches without consideration of ambiguities.

Usage

```
estimate.cvg(lex.df, k = 18, mode.directionality, sample = "")
```

Arguments

k	A numeric giving the size of the primers.
mode.directionality	Estimation of coverage for forward/reverse/both?
sample	An optional identifier for the sample.
seqs	A character vector of sequences to evaluate coverage for.

Value

A list with entries fw and rev giving data frames for forward/reverse binding.

`estimate.cvg.dir` *Estimation of Primer Coverage.*

Description

Estimates the possible coverage of primers using probes of size `k` and only considering perfect matches without consideration of ambiguities.

Usage

```
estimate.cvg.dir(seqs, k, id = "")
```

Arguments

`seqs`	A character vector of sequences to evaluate coverage for.
`k`	A numeric giving the size of the primers.
`id`	An optional identifier for the primers.

Value

A data frame with binding information.

`eval.comparison.primers`
Evaluation of Primers for Comparison

Description

Evaluate multiple primer sets according to the input constraint settings.

Usage

```
eval.comparison.primers(primer.data, constraint.settings)
```

Arguments

`primer.data`	List with primer data frames.
`constraint.settings`	List with constraint.settings.

Value

List with evaluated primer data frames.

`eval.constraints` *Evaluation of Constraints'*

Description

Evaluates whether the given primer data frame fulfills the required conditions.

Usage

```
eval.constraints(
  constraint.df,
  constraint.settings,
  active.constraints,
  mode.directionality = c("fw", "rev", "both"),
  primer.df
)
```

Arguments

`constraint.df`	Primer data frame with computed constraints.
`constraint.settings`	List with allowed values pers constraint.
`active.constraints`	Names of constraints to be evaluated.
`mode.directionality`	Directionality of primers
`primer.df`	Primer data frame corresponding to `constraint.df`.

Details

Constraint values should be contained in `constraint.df`. For each constraint in `active.constraints`, a boolean column with the name `EVAL_<constraint_name>` is generated, which indicates whether a primer in a given rows fulfills a constraint or not.

Value

Augments the `constraint.df` data frame with evaluation columns.

`evaluate.basic.cvg` *Evaluation of Primer Coverage.*

Description

Evaluates the coverage of a set of primers.

Usage

```
evaluate.basic.cvg(
  template.df,
  primers,
  mode.directionality = c("fw", "rev", "both"),
  allowed.mismatches,
  allowed.other.binding.ratio,
  allowed.region.definition = c("within", "any"),
  updateProgress = NULL
)
```

Arguments

`template.df`	Template data frame.
`primers`	Primer data frame.
`mode.directionality`	Primer directionality.
`allowed.mismatches`	The number of allowed mismatches per binding event.
`allowed.other.binding.ratio`	Ratio of primers that are allowed to bind to non-allowed regions. If `allowed.other.binding.ratio` >0 primers are allowed to bind at any location within the templates. However, a warning is given if the ratio of primers binding to non-target regions exceeds the `allowed.other.binding.ratio`.
`allowed.region.definition`	Definition of the target binding sites used for evaluating the coverage. If `allowed.region.definition` is `within`, primers have to lie within the allowed binding region. If `allowed.region.definition` is `any`, primers have to overlap with the allowed binding region. The default is that primers have to bind within the target binding region.
`updateProgress`	Progress callback function for shiny.

Value

Primer data frame with information on the covered template sequences.

evaluate.constrained.cvg *Evaluation of Primer Coverage.*

Description

Evaluates the coverage of a set of primers.

Usage

```
evaluate.constrained.cvg(
  template.df,
  primer.df,
  cvg.df,
  mode.directionality = c("fw", "rev", "both"),
  settings,
  updateProgress = NULL
)
```

Arguments

template.df	Template data frame.
primer.df	Primer data frame.
cvg.df	Data frame with basic coverage entries.
mode.directionality	Primer directionality.
settings	A DesignSettings object.
updateProgress	Progress callback function for shiny.

Value

Primer data frame with information on the covered template sequences.

evaluate.cvg *Evaluation of Coverage.*

Description

Evaluates primer coverage.

Usage

```
evaluate.cvg(
  template.seqs,
  primers,
  mode.directionality = c("fw", "rev"),
  allowed.mismatches,
  updateProgress = NULL
)
```

Arguments

`template.seqs`	Template sequences as a `DNAStringSet`.
`primers`	Primer sequences as a `DNAStringSet`.
`mode.directionality`	Directionality of primres
`allowed.mismatches`	Allowed number of mismatches between a primer and a template.
`updateProgress`	Progress function for shiny

Value

IRanges object with primer coverage information.

`evaluate.diff.primer.cvg` *Evaluation of Coverage.*

Description

Re-evaluates the coverage of primers under exclusion of certain templates.

Usage

```
evaluate.diff.primer.cvg(primers, excluded.seqs, template.df)
```

Arguments

`primers`	Primer data frame.
`excluded.seqs`	Identifiers of templates to be excluded.
`template.df`	Template data frame

Details

This function requires that `primers` was already annotated with primer coverage before.

Value

Primer data frame with updated coverage under the exclusion of `excluded.seeqs`.

evaluate.fw.rev.combinations *Evaluation of Set Combinations*

Description

Evaluates the combinations of forward and reverse primer sets.

Usage

```
evaluate.fw.rev.combinations(opti.fw, opti.rev, compatible.df, template.df)
```

Arguments

opti.fw	List with forward optimal primer sets.
opti.rev	List with reverse optimal primer sets.
compatible.df	Data frame containing the indicices of temperature-compatible forward and reverse primers sets.
template.df	Template data frame for which primers were designed.
opti.rev.indices	Indices for accessing opti.rev.

Value

List with information on the combinations of forward and reverse primers as well as the combined data frames themselves.

evaluate.GC.clamp *GC clamp*

Description

Determines the number of consecutive G/Cs at the 3' end.

Usage

```
evaluate.GC.clamp(y)
```

Arguments

y	Pirmer sequence from 5' to 3'.

Value

The length of the GC clamp.

`evaluate.primer.cvg` *Evaluation of Primer Coverage.*

Description

Evaluates the coverage of a set of primers.

Usage

```
evaluate.primer.cvg(
  template.df,
  primers,
  mode.directionality = c("fw", "rev", "both"),
  settings,
  updateProgress = NULL
)
```

Arguments

`template.df`	Template data frame.
`primers`	Primer data frame.
`mode.directionality`	Primer directionality.
`settings`	A `DesignSettings` object.
`updateProgress`	Progress callback function for shiny.

Value

Primer data frame with information on the covered template sequences.

`evaluate.template.constraints` *Evaluation of Template Constraints.*

Description

Evaluates the input template constraints.

Usage

```
evaluate.template.constraints(
  constraint.values,
  constraint.settings,
  active.constraints,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

`constraint.values`
: Data frame with template constraints

`constraint.settings`
: List specifying the allowed values for constraint evaluation.

`active.constraints`
: Strings specifying the constraints to check.

`mode.directionality`
: Direction of primers.

Value

List indicating which template constraints where fulfilled or not (TRUE/FALSE).

`exclude.cols` *Exclusion of Columns*

Description

Removes columns from a data frame.

Usage

```
exclude.cols(excl.col, template.df)
```

Arguments

`excl.col`	Names of columns in `template.df` to be removed.
`template.df`	Data frame for which columns in `excl.col` should be removed.

Value

`template.df` with removed columns as specified in `excl.col`.

filter.by.constraints *Filter By Constraints*

Description

Remove primers that do not fulfill the current constraints (evaluate all primers).

Usage

```
filter.by.constraints(
  filtered.df,
  constraint.df,
  current.constraints,
  active.constraints,
  mode.directionality = c("fw", "rev", "both"),
  template.df
)
```

Arguments

filtered.df	Primer data frame.
constraint.df	Data frame with constraint values.
current.constraints	List with constraint settings.
active.constraints	Strings giving the names of active constraints.
mode.directionality	Direction of primers
template.df	Template data frame.

Value

A list containing the filtered primer data frame, as well as a data frame of the excluded primers and the used filtering settings.

filter.comparison.primers *Filter Multiple Primer Sets.*

Description

Filters multiple primer sets at once.

Usage

```
filter.comparison.primers(
  primers,
  templates,
  active.constraints,
  settings,
  updateProgress = NULL
)
```

Arguments

primers	List with primer data frames.
templates	List with template data frames.
active.constraints	Strings giving the constraints that are to be checked.
settings	List with settings.
updateProgress	Progress callback function for shiny.

Value

A list with filtered primer data frames.

---

filter.primer.candidates

Filtering of Primer Candidates

Description

Filters primer candidates according to length and duplications.

Usage

```
filter.primer.candidates(primer.candidates, min.len)
```

Arguments

primer.candidates	Alignment of candidate primers.
min.len	Minimal required length of primers.

Value

Filtered alignment of candidate primers.

`filter.primer.set.opti` *Filtering of Primers*

Description

Filters a primer set during the optimization procedure.

Usage

```
filter.primer.set.opti(
  primer.df,
  sample,
  template.df,
  settings,
  mode.directionality,
  required.cvg,
  results.loc,
  target.temps
)
```

Arguments

`primer.df`	Primer data frame.
`sample`	Name of the current template sample.
`template.df`	Template data frame.
`settings`	List with settings for the constraints to be used for filtering.
`mode.directionality`	Primer direction.
`required.cvg`	Required ratio of covered templates. If `required.cvg` is set to 0, the constraints are not relaxed.
`results.loc`	Path to a directory where the results should be written.
`target.temps`	Target melting temperature of the primers in Celsius. This argument is only required if we try to match the melting temperatures of another primer set, e.g. when first optimizing forward and then optimizing reverse primers.

Value

The filtered primer data frame with respect to `required.cvg`.

filterLimits *Getter for Filtering Constraint Limits.*

Description

Gets the limits on the constraints that are used for the filtering procedure when designing primers using the `Input_Constraint_Boundaries` slot of the provided `DesignSettings` object `x`.

Usage

```
filterLimits(x)

## S4 method for signature 'DesignSettings'
filterLimits(x)
```

Arguments

`x`	A `DesignSettings` object.

Value

Gets the list of filtering limits.

filters *Getter for Filtering Constraints.*

Description

Gets the constraints on the physicochemical properties that are used for the filtering procedure when designing primers using the `Input_Constraints` slot of the provided `DesignSettings` object `x`.

Usage

```
filters(x)

## S4 method for signature 'DesignSettings'
filters(x)
```

Arguments

`x`	A `DesignSettings` object.

Value

Gets the list of filtering constraints.

filter_primers.by.Tm.delta

Filter by Melting Temperature Difference

Description

Filters primers by melting temperature differences.

Usage

```
filter_primers.by.Tm.delta(target.temp, selected.primers, max.Tm.delta)
```

Arguments

target.temp	Target melting temperature in Celsius.
selected.primers	Current candidate primer data frame.
max.Tm.delta	Maximum allowed difference of primer melting temperatures to target temperature.

Value

Filtered primer data frame.

fix_constraint_boundaries

Correction of Constraint Boundaries.

Description

Fixes the constraint boundaries if they are more narrow than the current settings.

Usage

```
fix_constraint_boundaries(constraints, constraint.limits, fix.limit = TRUE)
```

Arguments

constraints	A list with constraint settings.
constraint.limits	A list with constraint limits.
fix.limit	Whether the constraint limits should be adjusted. If FALSE, the constraint settings are adjusted.

Value

The corrected constraint limits.

format.constraints *Format Constraint Names.*

Description

Formats constraint names for frontend output.

Usage

```
## S3 method for class 'constraints'
format(constraints)
```

Arguments

constraints	The character vector of constraints to transform.

Value

A character vector with formatted constraint names.

format.seq.ali *Format mismatches*

Description

Formats a sequence for highlighting mismatches in an alignment.

Usage

```
## S3 method for class 'seq.ali'
format(seq, pos, format.type)
```

Arguments

seq	The input sequence.
pos	The mismatch positions to be formatted.
format.type	Vector of giving the style (bold/italics) for each pos.

Value

The input sequence with highlighted mismatch positions.

`format.seqs.tex` *Format a Sequence for LateX output.*

Description

Formats a sequence for LateX report output.

Usage

```
## S3 method for class 'seqs.tex'
format(seqs)
```

Arguments

`seqs`	Character vector of sequences.

Value

Formatted sequences.

`get.3prime.mismatch.pos` *Identification of 3' Mismatches.*

Description

Computes the lastmost position of a 3' mismatches of a primer with a template.

Usage

```
get.3prime.mismatch.pos(primers, mismatches)
```

Arguments

`primers`	Primer sequence strings.
`mismatches`	Comma-separated strings containing the primer mismatch positions.

Value

The closest position of a mismatch relative to the 3' end of the primer. Here, 1 indicates the terminal position, 2 the penultimate position, and so on. No mismatch is indicated by an infinite value.

get.analysis.mode *Direction of Primers.*

Description

Identifies the directionality of the input primers.

Usage

```
get.analysis.mode(primers)
```

Arguments

primers	A primer data frame.

Value

both if both, forward and reverse primers exist in primers. Otherwise, if either only forward primers or reverse primers exist, returns fw or rev, respectively.

get.consensus.seq *Computation of Consensus.*

Description

Computes the consensus of the sequences in the input alignment.

Usage

```
get.consensus.seq(ali)
```

Arguments

ali	An alignment object.

Value

A consensus sequence without gap characters.

get.constraint.value.idx *Retrive Constraint Indices.*

Description

Gets the index of the required constraint columns in the primer data frame.

Usage

```
get.constraint.value.idx(active.constraints, constraint.df)
```

Arguments

active.constraints	The names of the constraints for which to find the indices in constraint.df.
constraint.df	The primer data frame where the active.constraints should be found.

Value

Indices of active.constraints in constraint.df.

get.constraint.values *Get the Values of a Constraint.*

Description

Get the Values of a Constraint.

Usage

```
get.constraint.values(con.name, cur.candidates, mode.directionality)
```

Arguments

con.name	The name of the constraint.
cur.candidates	The Primers data frame where the values should be retrieved.
mode.directionality	The direction for which values should be retrieved.

Value

The constraint values corresponding to con.name for the primers cur.candidates.

get.coverage.matrix *Coverage Matrix*

Description

Constructs a coverage matrix where rows indicate templates and columns indicate primers.

Usage

```
get.coverage.matrix(primer.df, template.df, constraints = NULL)
```

Arguments

primer.df	Primer data frame.
template.df	Template data frame.
constraints	A character vector of coverage constraints to be used as entries for the coverage matrix instead of the 0/1 encoding. At its default setting (NULL), the 0/1 encoding is used.

Details

Entry (i,j) in the matrix is equal to 1 if primer j covers template i and otherwise 0.

Value

The binary coverage matrix.

get.covered.templates *Covered Templates*

Description

Get the indices of covered templates.

Usage

```
get.covered.templates(Tm.set, template.df)
```

Arguments

Tm.set	Primer data frame.
template.df	Template data set.

Value

Index of templates that are covered by the primers in Tm.set.

get.cross.dimers *Cross dimers*

Description

Computes all possible primer cross-dimers.

Usage

```
get.cross.dimers(
  primers.1,
  primers.2,
  ions,
  annealing.temp,
  check.idx = NULL,
  no.structures = FALSE,
  mode = c("symmetric", "asymmetric")
)
```

Arguments

primers.1	Input primers.
primers.2	Input primers.
ions	Sodium-equivalent ionic concentration.
annealing.temp	The PCR annealing temperature.
check.idx	indices of primers for checking cross-dimerization
no.structures	Whether to compute structures of dimers.
mode	'symmetric', if primers.1 and primers.2 carry the same information (i.e. fw-fw, rev-rev, fw-rev), 'asymetric' else.

Value

Data frame with potential cross dimers.

get.cvg.constraint.settings *Gather all Coverage Constraints.*

Description

Constructor for coverage constraint settings.

Usage

```
get.cvg.constraint.settings(
  allowed.stop.codons,
  allowed.efficiency,
  disallowed.mismatch.pos,
  allowed.anneal.deltaG,
  allowed.substitutions,
  allowed.coverage.model
)
```

Arguments

`allowed.stop.codons`
: Whether mismatch binding events inducing stop codons in the amino acid sequence are allowed.

`allowed.efficiency`
: Min/max for primer efficiency.

`disallowed.mismatch.pos`
: The positions from the 3' terminal end of primers where mismatches shall be prevented.

`allowed.anneal.deltaG`
: Maximal allowed free energy of template-primer annealing.

`allowed.substitutions`
: Whether mismatch binding events inducing substitutions in the amino acid sequence are allowed.

Value

List with all coverage constraint settings.

`get.cvg.gain` *Computation of Coverage Gain.*

Description

Computes the coverage gain from `covered.seqs`.

Usage

```
get.cvg.gain(
  covered.seqs,
  template.df,
  missing.df,
  candidate.df,
  con.names,
  constraint.limits,
  feasible.only = FALSE
)
```

Arguments

covered.seqs	List with covered sequences.
template.df	A Templates data frame.
missing.df	A Templates data frame containing only the templates that still need to be covered.
candidate.df	A Primers data frame containing candidate primers.
con.names	The constraint to evaluate the coverage gain for upon being relaxed.
constraint.limits	A list with constraint limits.
feasible.only	Whether only feasible coverage gains are to be outputed. Here, *feasible* relates to coverage gains that can be obtained directly with the next relaxation.

Value

The number of covered sequences to be gained.

---

get.delta.G *Change in Free Energy.*

Description

Computes the change in free energy.

Usage

```
get.delta.G(delta.H, delta.S, temp = 37)
```

Arguments

delta.H	Change in enthalpy in cal/mol.
delta.S	Change in entropy cal/mol*K.
temp	Temperature in Celsius for which to compute free energy change.

Value

The change in free energy in kcal/mol.

get.dimer.data *Retrieval of dimerization energies.*

Description

Uses OligoArrayAux to compute dimerization candidates.

Usage

```
get.dimer.data(s1, s2, annealing.temp, ions, no.structures)
```

Arguments

s1	Nucleotide character vectors (5' to 3')
s2	Nucleotide character vectors (5' to 3')
annealing.temp	The PCR annealing temperature in Celsius.
ions	The sodium-equivalent ions used in the PCR.
no.structures	Whether to compute structures of dimers.

Value

A data frame containing free energies in the field DeltaG and the dimerization structure in Structure.

get.duplex.energies *Determination of the Free Binding Energy.*

Description

Computest the free energy of annealing between primers and templates. If the mode is set to "on_target", the free energies of binding events in the allowed region are computed, while if the mode is set to "off_target", the free energies of off-target events are computed.

Usage

```
get.duplex.energies(
  primer.df,
  template.df,
  annealing.temp,
  settings,
  mode = c("on_target", "off_target")
)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.
annealing.temp	The vector of optimal annealing temperatures of the primers.
settings	A DesignSettings object.
mode	If the mode is set to "on_target", the free energies of binding events in the allowed region are computed, while if the mode is set to "off_target", the free energies of off-target events are computed.

Value

A list of lists containing the numeric free energies of the annealing events for every primer.

---

get.eval.cols *Retrieval of Evaluation Columns.*

Description

Retrieves the evaluation columns by intersecting the already evaluated constraints in primer.data as well as the constraints specified in input constraint settings.

Usage

```
get.eval.cols(primer.data, constraint.settings)
```

Arguments

primer.data	A list with Primers objects.
constraint.settings	A list with constraint settings.

Value

A character vector with EVAL-columns.

get.extension *Identification of File extension.*

Description

Identifies the file extension of x.

Usage

```
get.extension(x)
```

Arguments

x	A string for a filename.

Value

The extension of x.

get.ILP.vars *Retrieval of ILP Decisions*

Description

Retrieves ILP decision variables.

Usage

```
get.ILP.vars(ILP, original.dim = NULL)
```

Arguments

ILP	A solved ILP instance.
original.dim	Dimension of ILP before using presolve.

Details

The original dimension of the ILP is required to determine the correct decisions when presolve has been active and dimensions of the ILP might have changed.

Value

The ILP decision variables.

`get.init.file.name` *File Name for Initialized Primers.*

Description

Constructs a filename for initialized primers.

Usage

```
get.init.file.name(
  cur.results.loc,
  GROUP,
  primer.lengths,
  mode.directionality,
  allowed.region.definition,
  init.algo,
  max.degen,
  conservation
)
```

Arguments

`cur.results.loc`	Directory where the file should be stored.
`GROUP`	Sample name of templates.
`primer.lengths`	Interval of desired primer lengths.
`mode.directionality`	Directionality of the primers
`allowed.region.definition`	Definition of the allowed region.
`init.algo`	Initialization algorithm identifier.
`max.degen`	Maximum degeneracy of primers.
`conservation`	Required ratio of primer conservation.

Value

A filename for the initialized primers.

get.leader.exon.regions
Assign Binding Regions

Description

Augments a template data frame with individual binding regions.

Usage

```
get.leader.exon.regions(lex.seqs, uni.leaders)
```

Arguments

lex.seqs	Data frame with template sequences.
uni.leaders	Data frame with individual allowed binding regions.

Value

Template data frame with annotated binding regions.

get.leader.exon.regions.single
Individual Binding Annotation

Description

Annotate individual binding regions.

Usage

```
get.leader.exon.regions.single(
  l.seq,
  lex.seq,
  direction = c("fw", "rev"),
  gap.char
)
```

Arguments

l.seq	Data frame with individual binding regions.
lex.seq	Template data frame.
direction	The primer direction for which the binding info is valid.
gap.char	The character for gaps in alignments.

Value

Template data frame with annotated binding regions.

`get.matches` *Identification of Sequence Matches.*

Description

Identifies matches between two strings provided by OligoArrayAux.

Usage

```
get.matches(s1, s2)
```

Arguments

`s1`	The aligned nucleotide sequence character vector.
`s2`	The aligned, matching substring of `s1`.

Value

A match vector (`M` for matches, `X` for mismatches).

`get.melting.temp.diff` *Computation of Maximal Melting Temperature Differences.*

Description

Computation of Maximal Melting Temperature Differences.

Usage

```
get.melting.temp.diff(Tm.fw, Tm.rev)
```

Arguments

`Tm.fw`	The melting temperatures of forward primers.
`Tmr.rev`	The melting temperatures of reverse primers.

Value

The worst-case melting temperature difference, for every primer.

get.merge.idx *Indices for merging sequences*

Description

Identifies the indices of similar input sequences to be merged.

Usage

```
get.merge.idx(seqs, max.degeneracy)
```

Arguments

seqs	The input sequence strings.
max.degeneracy	The maximal allowed degeneracy of a merged seq.

Value

A list of lists containing the indices of seqs to be merged. For example [[1,2,3]] would indicate to merge primers 1, 2, and 3.

get.missing.df *Uncovered Templates.*

Description

Computes a data frame containing the templates that are not yet covered.

Usage

```
get.missing.df(
  filtered.df,
  template.df,
  Tm.brackets,
  settings,
  mode.directionality
)
```

Arguments

filtered.df	An object of class Primers.
template.df	An object of class Templates.

Value

A Templates data frame containing the missing templates.

get.ORFs *Identification of ORFs.*

Description

Given a template data frame, identify the exon reading frames in the sequences.

Usage

```
get.ORFs(template.df)
```

Arguments

template.df	template data frame.

Value

Returns a data frame containing the shift of the ORF (either 0,1, or 2) for every sequence, as well as a comment in case of problems.

get.other.constraint.settings *Gather all Other Constraints (for Shiny frontend).*

Description

Constructor for other constraint settings (non-PCR, non-filtering, non-optimization).

Usage

```
get.other.constraint.settings(
  allowed_mismatches,
  allowed_other_binding_ratio,
  allowed_region_definition
)
```

Arguments

allowed_mismatches	Allowed mismatches for primers binding events.
allowed_other_binding_ratio	Ratio of primers allowed to bind to non-target regions.
allowed_region_definition	The definition of the allowed region.

Value

List with all other constraint settings.

`get.PCR.settings` *Gather all PCR settings.*

Description

Gathers all PCR settings (e.g. for XML output).

Usage

```
get.PCR.settings(
  use_taq_polymerase,
  annealing_temp,
  Na_concentration,
  Mg_concentration,
  K_concentration,
  Tris_concentration,
  primer_concentration,
  template_concentration,
  nbr.cycles
)
```

Arguments

`annealing_temp`	Annealing temperature in Celsius.
`Na_concentration`	Sodium ion concentration.
`Mg_concentration`	Magensium ion concentration.
`K_concentration`	Potassium ion concentration.
`Tris_concentration`	Tris buffer concentration.
`primer_concentration`	Primer concentration.
`template_concentration`	Template concentration.

Value

List with all PCR settings.

get.plot.height *Plot Extent*

Description

Returns the extent of a plot.

Usage

```
get.plot.height(N, px.per.n = 50, min.size = 300, max.size = 1500000)
```

Arguments

N	Number of observations to plot.
px.per.n	Pixels required per observations.
min.size	Minimal extent of plot in pixels.
max.size	Maximal extent of plot in pixels.

Value

The extent of the plot.

get.primer.binding.idx *Retrieval of Allowed Binding Indices.*

Description

Retrieves the indices of allowed binding events in binding for the primer with index x and type primer.type.

Usage

```
get.primer.binding.idx(
  binding,
  primer.type = c("fw", "rev", "both"),
  x,
  allowed.other.binding.ratio
)
```

Arguments

binding	IRanges binding information.
primer.type	Direction of primer.
x	Index of primer in the primer data frame.
allowed.other.binding.ratio	The ratio of allowed off-target binding events.

Value

Indices in binding for primer with index codex that are allowed.

`get.primer.identifier.string` *Primer Identifier Creation.*

Description

Creates identifiers for generated primers.

Usage

```
get.primer.identifier.string(
  sample,
  seq.IDs,
  seq.identifier,
  all.starts,
  all.ends,
  identifier,
  seq.primers
)
```

Arguments

`sample`	Sample name of the templates.
`seq.IDs`	Identifiers of the templates.
`all.starts`	Primer positions (start).
`all.ends`	Primer positions (end).
`identifier`	Direction keyword.
`seq.primers`	The primer sequences as strings.
`seq.identifiers`	The index of the seq.

Value

Identifiers for each primer.

`get.redundant.cols` *Identification of Redudant Primers.*

Description

Identifies primers that are redundant.

Usage

```
get.redundant.cols(cvg.matrix)
```

Arguments

`cvg.matrix`	Binary matrix of coverage events.

Details

Redundant primers do not reduce the coverage when removed.

Value

TRUE for redundant primers, FALSE otherwise.

`get.relative.binding.pos` *Retrieval of Relative Binding Positions.*

Description

Retrieves primer binding position relative to allowed regions of either forward or reverse primers, as specified by `direction`.

Usage

```
get.relative.binding.pos(allowed, primer.pos, direction, covered.seqs.idx)
```

Arguments

`allowed`	Positions where binding is allowed in the templates.
`primer.pos`	Binding position of primer (absolute).
`direction`	Direction (either fw/rev).
`covered.seqs.idx`	Indices of covered templates.

Value

Numeric of relative binding position to allowed region.

get.run.names *Getter for Run Names.*

Description

Retrieves the run names of the input data.

Usage

```
get.run.names(primer.data)
```

Arguments

primer.data	A list with Primers or Templates.

Value

A vector with identifiers for every set.

get.self.dimers *Self dimerization*

Description

Computes possible self-dimers.

Usage

```
get.self.dimers(
  primers.1,
  primers.2,
  ions,
  annealing.temp,
  no.structures = FALSE
)
```

Arguments

primers.1	Input primers
primers.2	(Copy/reverse) of the input primers
ions	Sodium-equivalent ionic concentration.
annealing.temp	The annealing temperature.
no.structures	Whether the dimerization structure shall be computed.

Value

Possible self-dimer conformations.

get.sets.from.decisions *Optimal Sets from Decision Variables*

Description

Determines primer sets from decision variables from ILP.

Usage

```
get.sets.from.decisions(ILP.df, Tm.sets)
```

Arguments

ILP.df	Data frame with ILP optimization results.
Tm.sets	List with primer data frames for every target melting temperature.

Value

A list with optimal primer data sets for every target temperature.

get.static.tool.info *Retrieval of Tool Information.*

Description

Constructs a data frame containing information about the tools.

Usage

```
get.static.tool.info()
```

Value

A data frame with information about the required tools.

get.tree.seqs *Determine Tree Consensus Sequences*

Description

Creates all possible consensus sequences from a phylogenetic tree.

Usage

```
get.tree.seqs(tree, max.degen, primer.candidates)
```

Arguments

tree	The phylogenetic tree.
max.degen	The maximal degeneration of consensus primers.
primer.candidates	Alignment of primers.

Details

Ambiguous sequences are only generated with a degeneracy of at most max.degen. The tree is iterated from leaves to the top, i.e., starting from least degeneracy to most degeneracy. Merges only take place when the degeneracy of the resulting sequence would be at most max.degen. Gaps are removed from the alignments.

Value

Data frame with consensus primers extracted from the tree.

get.unlist.idx *Index for Unlisting.*

Description

Determines indices for unlisting.

Usage

```
get.unlist.idx(primer.start, primer.data.idx)
```

Arguments

primer.start	Numeric vector.
primer.data.idx	Selection indices.

Value

Indices.

---

get_constraint_deviation_data *Retrieve data for Constraint Deviations.*

Description

Retrieve data for Constraint Deviations.

Usage

```
get_constraint_deviation_data(constraint.df, constraint.settings)
```

Arguments

constraint.df	An evaluated object of class Primers.
constraint.settings	A list with settings for the constraints that are to be evalated.

Value

A data frame providing primer-specific information on deviations of primer properties from the desired properties.

---

get_covered.vanilla *Determination of the Covered Sequences.*

Description

Determines the covered template sequences given by template.df that are covered by the primers given by primers.

Usage

```
get_covered.vanilla(primers, template.df, mode.directionality = NULL)
```

Arguments

primers	A Primers object containing the primers for which the coverage should be evaluated.
template.df	A Templates object containing the template sequences corresponding to primers.
mode.directionality	If mode.directionality is provided, the coverage of templates is computed for a specific direction of primers. Either "fw" (forward coverage only), "rev" (reverse coverage only), or "both" for both directions. If mode.directionality is not provided the direction is determined by the input primers.

Details

The manner in which the coverage ratio is evaluated depends on the directionality of the input primers. If either only forward or reverse primers are inputted, the individual coverage of each primer is used to determine the overall coverage. If, however, forward and reverse primers are inputted at the same time, the coverage is defined by the intersection of binding events from both, forward and reverse primers.

Value

The IDs of all covered templates.

---

get_cvg_stats,list-method

Coverage Statistics for Multiple Primer Sets.

Description

Retrieve statistics on covered templates for multiple primer sets.

Usage

```
## S4 method for signature 'list'
get_cvg_stats(
  primers,
  templates,
  for.viewing = FALSE,
  total.percentages = FALSE,
  allowed.mismatches = Inf,
  cvg.definition = c("constrained", "basic")
)
```

Arguments

`primers`	A list with objects of class `Primers` containing primers with evaluated coverage.
`templates`	A list with objects of class `Templates` containing templates with evaluated coverage.
`for.viewing`	Whether the table should be formatted for viewing rather than processing.
`total.percentages`	Whether group coverage percentages should relate to all template sequences or just those templates belonging to a specific group.
`allowed.mismatches`	The maximal allowed number of mismatches. By default, the number of mismatches is not restricted.
`cvg.definition`	If `cvg.definition` is set to "constrained", the statistics for the expected coverage (after applying the coverage constraints) are retrieved. If `cvg.definition` is set to "basic", the coverage is determined solely by string matching (i.e. without applying the coverage constraints). By default, `cvg.definition` is set to "constrained".

Value

Data frame with coverage statistics.

`get_cvg_stats,Primers-method`

Coverage Statistics of a Primer Set.

Description

Retrieve statistics on the templates that are covered by a primer set.

Usage

```
## S4 method for signature 'Primers'
get_cvg_stats(
  primers,
  templates,
  for.viewing = FALSE,
  total.percentages = FALSE,
  allowed.mismatches = Inf,
  cvg.definition = c("constrained", "basic")
)
```

Arguments

`for.viewing`	Whether the table should be formatted for viewing rather than processing.
`total.percentages`	Whether group coverage percentages should relate to all template sequences or just those templates belonging to a specific group.
`allowed.mismatches`	The maximal allowed number of mismatches. By default, the number of mismatches is not restricted.
`cvg.definition`	If `cvg.definition` is set to "constrained", the statistics for the expected coverage (after applying the coverage constraints) are retrieved. If `cvg.definition` is set to "basic", the coverage is determined solely by string matching (i.e. without applying the coverage constraints). By default, `cvg.definition` is set to "constrained".
`primer.df`	An object of class `Primers` containing primers with evaluated coverage.
`template.df`	An object of class `Templates` containing templates with evaluated coverage.

Value

Data frame with coverage statistics.

get_max_set_coverage *Determination of Maximal Coverage.*

Description

Determines the maximal coverage ratio of a set of primers for primer subsets valid for a certain temperature range. a certain melting temperature range.

Usage

```
get_max_set_coverage(
  primer.df,
  template.df,
  Tm.brackets,
  settings,
  mode.directionality,
  max.only = TRUE
)
```

Arguments

primer.df	An object of class Primers.
template.df	An objectc of class Templates.
Tm.brackets	A data frame with temperature information.
settings	A DesignSettings object.
mode.directionality	The direction of the primers.
max.only	Whether only the maxium coverage shall be returned. If max.only is FALSE, the coverage ratios of all melting temperature sets according to Tm.brackets are returned.

Value

The maximal coverage ratio of a primer set if max.only is TRUE or the coverages of all melting temperature seets if max.only is FALSE.

get_plot_primer_data *Data for Primer Plot.*

Description

Constructs a data frame containing information about primer binding events.

Usage

```
get_plot_primer_data(
  primer.df,
  template.df,
  identifier = NULL,
  relation = c("fw", "rev")
)
```

Arguments

primer.df	An object of class Primers containing primers with evaluated primer coverage.
template.df	An object of class Templates with template sequences corresponding to primer.df.
identifier	Identifiers of primers that are to be considered. If identifier is set to NULL (the default), all primers are considered.
relation	Compute binding positions relative to forward (fw) or reverse (rev) binding regions. The default is "fw".

Value

Data frame with primer binding data.

---

get_primer_cvg_mm_plot_df

Data for Mismatch Primer Coverage Plot.

Description

Ensures that there's an entry for every possible mismatch setting.

Usage

```
get_primer_cvg_mm_plot_df(primer.df, template.df)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.

Value

A data frame for plotting mismatch primer coverage.

get_report_fname *Creation of a Filename for Reports.*

Description

Creates the filename for reports.

Usage

```
get_report_fname(report.name, sample.name)
```

Arguments

report.name	The identifier for the report type.
sample.name	The identifier of the sample that was analyzed.

Value

A character vector.

get_template_cvg_data *Retrieval of Template Coverage Data.*

Description

Determines the coverage of the templates for individual allowed mismatch settings and coverage definitions.

Usage

```
get_template_cvg_data(primer.df, template.df)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.

Value

Computes a data frame providing the coverage of the templates for the basic as well as expected (constrained) coverage.

hclust.tree *Hierarchical Clustering.*

Description

Performs hierarchical clustering on aligned primer sequences.

Usage

```
hclust.tree(primer.candidates)
```

Arguments

primer.candidates	Alignment of primer candidates.

Details

The clustering is performed to identify similar groups of primer candidates that can be merged to form degenerate primers.

Value

Phylogeny of the input `primer.candidates`.

highlight.mismatch *Highlight mismatches*

Description

Collects information on the mutations present in the input and highlights the mutations in the sequence.

Usage

```
highlight.mismatch(seq, mm.seq)
```

Arguments

seq	character vector of the original sequence
mm.seq	character vector of the mutated sequence

Value

A list highlighting the mutations and additional information (mutation type, number, etc.)

html.format.structure *Formats a Dimerization Structure for HTML.*

Description

Formats a Dimerization Structure for HTML.

Usage

```
html.format.structure(structures)
```

Arguments

structures	A character vector of dimerization structures.

Value

HTML-formatted character vectors.

I.cvg *Primer Coverage.*

Description

Determines the indices of covered templates for every primer.

Usage

```
I.cvg(cvg.matrix)
```

Arguments

cvg.matrix	Binary matrix of covering events.

Value

A list with covered templates for every primer.

ILPConstrained *Construct Coverage ILP.*

Description

Constructs an ILP modeling the primer set cover problem.

Usage

```
ILPConstrained(D, cvg.matrix, time.limit = NULL, presolve.active = FALSE)
```

Arguments

D	Binary dimerization matrix.
cvg.matrix	Binary coverage matrix.
time.limit	Time limit for ILP optimization in seconds.
presolve.active	Whether the ILP presolver should be used. This is set to FALSE by default, since presolving may lead to inferior solutions. However, for large problems presolving might be useful.

Value

An instance of the set cover ILP.

initialize.primer.set *Creation of Initial Primers*

Description

Creates a set of candidate primers.

Usage

```
initialize.primer.set(
  template.df,
  sample.name,
  primer.lengths,
  allowed.region.definition,
  mode.directionality,
  init.algo,
  max.degen,
  conservation,
  cur.results.loc
)
```

Arguments

`template.df`	Template data frame.
`sample.name`	Name of the template sample.
`primer.lengths`	Interval of minimal and maximal desired primer length.
`allowed.region.definition`	Definition of the allowed binding region.
`mode.directionality`	Direction of primers to be created.
`init.algo`	Algorithm for initializing primers.
`max.degen`	Maximal allowed degeneration of created primers.
`conservation`	Required conservation of primers. The value of `conservation` should be in the range[0,1].
`cur.results.loc`	Location for writing the primers as csv.

Value

An initial primer data frame.

`Input` *Input Functionalities.*

Description

`read_primers` Reads one or multiple input files with primer sequences. The input can either be in FASTA or in CSV format.

`read_templates` Read one or multiple files with template sequences in FASTA or CSV format.

`read_settings` Loads primer analysis settings from an XML file.

`Templates` The `Templates` class encapsulates a data frame containing the sequencs of the templates, their binding regions, as well as additional information (e.g. template coverage).

`Primers` The `Primers` class encapsulates a data frame representing a set of primers. Objects of this class store all properties associated with a set of primers, for example the results from evaluating the properties of a primer set or from determining its coverage.

Usage

```
Templates(...)

read_templates(
  fname,
  hdr.structure = NULL,
  delim = NULL,
  id.column = NULL,
```

```
  rm.keywords = NULL,
  remove.duplicates = FALSE,
  fw.region = c(1, 30),
  rev.region = c(1, 30),
  gap.char = "-",
  run = NULL
)

Primers(...)

read_primers(
  fname,
  fw.id = "_fw",
  rev.id = "_rev",
  merge.ambig = c("none", "merge", "unmerge"),
  max.degen = 16,
  template.df = NULL,
  adapter.action = c("warn", "rm"),
  sample.name = NULL,
  updateProgress = NULL
)

read_settings(
 filename = list.files(system.file("extdata", "settings", package = "openPrimeR"),
    pattern = "*.xml", full.names = TRUE),
  frontend = FALSE
)
```

Arguments

`...`	A data frame fulfilling the structural requirements for initializing a `Templates` or `Primers` object.
`fname`	Character vector providing either a single or multiple paths to FASTA or CSV files.
`hdr.structure`	A character vector describing the information contained in the FASTA headers. In case that the headers of `fasta.file` contain template group information, please include the keyword "GROUP" in `hdr.structure`. If the numer of elements provided via `hdr.structure` is shorter than the actual header structure, the missing fields are ignored.
`delim`	Delimiter for the information in the FASTA headers.
`id.column`	Field in the header to be used as the identifier of individual template sequences.
`rm.keywords`	A vector of keywords that are used to remove templates whose headers contain any of the keywords.
`remove.duplicates`	Whether duplicate sequence shall be removed.
`fw.region`	The positional interval from the template 5' end specifying the binding sites for forward primers. The default `fw.region` is set to the first 30 bases of the templates.

`rev.region` The positional interval from the template 3' end specifying the binding sites for reverse primers. The default `rev.region` is set to the last 30 bases of the templates.

`gap.char` The character in the input file representing gaps. Gaps are automatically removed upon input and the default character is "-".

`run` An identifier for the set of template sequences. By default, `run` is `NULL` and its value is set via `template.file`.

`fw.id` For FASTA input, the identifier for forward primers in the FASTA headers.

`rev.id` For FASTA input, the identifier for reverse primers in the FASTA headers.

`merge.ambig` Indicates whether similar primers should be merged ("merge") using IUPAC ambiguity codes or whether primers should be disambiguated ("unmerge"). By default `merge.ambig` is set to "none", leaving primers as they are.

`max.degen` A scalar numeric providing the maximum allowed degeneracy for merging primers if `merge.ambig` is set to "merge". Degeneracy is defined by the number of disambiguated sequences that are represented by a degenerate primer.

`template.df` An object of class `Templates`. If `template.df` is provided for `read_primers` then the primers are checked for restriction sites upon input; otherwhise they are not checked.

`adapter.action` The action to be performed when `template.df` is provided for identifying adapter sequences. Either "warn" to issue warning about adapter sequences or "rm" to remove identified adapter sequences. The default is "warn".

`sample.name` An identifier for the input primers.

`updateProgress` A Shiny progress callback function. This is `NULL` by default such that no progress is tracked.

`filename` Path to a valid XML file containing the primer analysis settings. By default, `filename` is set to all settings that are shipped with openPrimeR and the lexicographically first file is loaded.

`frontend` Indicates whether settings shall be loaded for the Shiny frontend. In this case no unit conversions for the PCR settings are performed. The default setting is `FALSE` such that the correct units are used.

Details

In the following you can find a description of the most important columns that can be found in an object of class `Templates`. Note that angle brackets in the column names indicate the existence of multiple possibilities.

`ID` The identifiers of the templates.

`Identifier` The internal identifiers of the templates.

`Group` The identifiers of the groups that the templates belong to.

`Allowed_Start_<fw|rev>` The start of the interval in the templates where binding is allowed for forward and reverse primers, respectively.

`Allowed_End_<fw|rev>` The end of the interval in the templates where binding is allowed for forward and reverse primers, respectively.

`Allowed_<fw|rev>` The template sequence where binding is allowed for forward and reverse primers, respectively.

`Run` An identifier for the set of template sequences.

`Covered_By_Primers` The identifiers of primers covering the templates, when the template coverage has been annotated.

`primer_coverage` The number of primers covering the templates, when the template coverage has been annotated.

When loading a FASTA file with `read_templates`, the input arguments `hdr.structure`, `delim`, `id.column`, `rm.keywords`, `remove.duplicates`, `fw.region`, `rev.region`, `gap.character`, and `run` are utilized. Most importantly, `hdr.structure` and `delim` should match the FASTA header structure. To learn more about setting the primer binding regions, consider the `assign_binding_regions` function. In contrast, when a CSV file is loaded with `read_templates`, the data are loaded without performing any modifications because the CSV file should represent an object of class `Templates`, which can be stored using the `write_templates` function.

When loading primers via `read_primers`, the input arguments `fw.id`, `rev.id`, `merge.ambig`, and `max.degen` are only used for loading primers from a FASTA file. In this case, please ensure that `fw.id` and `rev.id` are set according to the keywords indicating the primer directionalities in the FASTA file. When loading primers from a CSV file, the format of the file should adhere to the structure defined by the `Primers` class.

When loading a settings file with `read_settings`, if `filename` is not provided, a default XMl settings file is loaded. Please review the function's examples to learn more about the default settings. If you want to load custom settings, you can store a modified `DesignSettings` object as an XML file using `write_settings`.

Value

The `Templates` constructor returns a `Templates` object, an instance of a data frame.

`read_templates` returns a single object of class `Templates` if a single filename was provided or a list of such objects if multiple file names were provided.

The `Primers` constructor returns an object of class `Primers`.

`read_primers` returns a single object of class `Primers` if a single input file is provided or a list of such objects if multiple files are provided.

`read_settings` returns an object of class `DesignSettings`.

Basic columns

In the following you can find a description of the most important columns that can be found in objects of class `Primers`. Note that angular brackets indicate the existence of multiple possibilities. The following columns are present when a set of primers is loaded from a FASTA file using `read_primers`:

`ID` The identifiers of the primers.

`Identifier` The internal identifiers of the primers.

`Forward` The sequences of forward primers.

`Reverse` The sequences of reverse primers.

`primer_length<fw|rev>` The lengths of forward and reverse primer sequences, respectively.

`Direction` Either 'fw' for forward primers, 'rev' for reverse primers, or 'both' for a primer pair.

`Degeneracy_<fw|rev>` The degeneracy (ambiguity) of forward and reverse primers, respectively.

`Run` An identifier describing the primer set.

Coverage-related columns

The following columns are only available in an object of class `Primers` after primer coverage has been computed, that is after `check_constraints` has been called with the active `primer_coverage` constraint. Computed coverage values relating solely to string matching are indicated by the prefix `Basic_`, while columns without this prefix relate to the coverage after applying the constraints formulated via `CoverageConstraints`. Information on off-target coverage events are indicated by the `Off_` prefix, while on-target coverage events do not carry this prefix.

`primer_coverage` The number of templates that are covered by the primers. Note that if a primer set contains primers of both directions, a template is only considered covered if it is covered by primers of both directions.

`Coverage_Ratio` The ratio of templates that are covered by the primers.

`Binding_Position_Start_<fw|rev>` The upstream position in the templates where forward and reverse primers respectively bind.

`Binding_Position_End_<fw|rev>` The downstream position in the templates where forward and reverse primers respectively bind.

`Relative_<Forward|Reverse>_Binding_Position_<Start|End>_<fw|rev>` The binding upstream (`Start`) or downstream (`End`) positions of the primers relative to the forward (`Forward`) or reverse (`Reverse`) binding regions, either for forward (`fw`) or reverse primers (`rev`).

`Binding_Region_Allowed` Whether a coverage event occurred in the target binding region or not. If the allowed off-target ratio was set to 0 only coverage events within the the target region are reported.

`Nbr_of_mismatches_<fw|rev>` The number of mismatches of forward and reverse primer coverage events, respectively.

`Mismatch_pos_<fw|rev>` The position of mismatches for forward and reverse coverage events, respectively. Mismatch positions are reported relative to the 3' end, that is, position 1 indicates a mismatch in the last base of a primer.

`primer_specificity` The specificity of a primer as determined by its ratio of off-target binding events.

Constraint-related columns

Each constraint that is considered when calling `check_constraints` gives rise to at least one column in the provided `Primers` object. Due to the large number of possible constraints, we will limit our description to the `gc_clamp` constraint. Once the GC clamp property has been computed, the `gc_clamp_fw` column contains the length of the GC clamp for forward primers and `gc_clamp_rev` the corresponding length for reverse primers. Whether the desired extent of the GC clamp was obtained by a primer is indicated by the `EVAL_gc_clamp` column. It contains `TRUE` when the GC clamp constraint was fulfilled and `FALSE` when it was broken. To identify whether all required constraints were fulfilled by a primer, the `constraints_passed` column can be used. It contains `TRUE` if all `active.constraints` used by `check_constraints` were fulfilled and `FALSE` otherwise.

Examples

```
# Load a set of templates:
fasta.file <- system.file("extdata", "IMGT_data", "templates",
     "Homo_sapiens_IGH_functional_exon.fasta", package = "openPrimeR")
hdr.structure <- c("ACCESSION", "GROUP", "SPECIES", "FUNCTION")
template.df <- read_templates(fasta.file, hdr.structure, "|", "GROUP")
# Load templates from a FASTA file
fasta.file <- system.file("extdata", "IMGT_data", "templates",
          "Homo_sapiens_IGH_functional_exon.fasta", package = "openPrimeR")
hdr.structure <- c("ACCESSION", "GROUP", "SPECIES", "FUNCTION")
template.df.fasta <- read_templates(fasta.file, hdr.structure, "|", "GROUP")
# Load mutliple FASTA files
fasta.files <- c(fasta.file, fasta.file)
template.df.fastas <- read_templates(fasta.files, hdr.structure, "|", "GROUP")
# Load templates from a previously stored CSV file
csv.file <- system.file("extdata", "IMGT_data", "comparison",
               "templates", "IGH_templates.csv", package = "openPrimeR")
template.df.csv <- read_templates(csv.file)
# Load multiple CSV files:
csv.files <- c(csv.file, csv.file)
template.df.csvs <- read_templates(csv.files)
# Load a mixture of FASTA/CSV files:
mixed.files <- c(csv.file, fasta.file)
template.data <- read_templates(mixed.files)

# Load a set of primers
primer.location <- system.file("extdata", "IMGT_data", "primers", "IGHV",
                     "Ippolito2012.fasta", package = "openPrimeR")
primer.df <- read_primers(primer.location, "_fw", "_rev")

primer.fasta <- system.file("extdata", "IMGT_data", "primers", "IGHV",
                     "Ippolito2012.fasta", package = "openPrimeR")
primer.df <- read_primers(primer.fasta, "_fw", "_rev")
# Read multiple FASTA files
fasta.files <- list.files(system.file("extdata", "IMGT_data", "primers",
                 "IGHV", package = "openPrimeR"), pattern = "*\\.fasta",
                 full.names = TRUE)[1:3]
primer.data <- read_primers(fasta.files)
# Read primers from a CSV file
primer.csv <- system.file("extdata", "IMGT_data", "comparison",
              "primer_sets", "IGL", "IGL_openPrimeR2017.csv",  package = "openPrimeR")
primer.df <- read_primers(primer.csv)
# Read multiple primer CSV files
primer.files <- list.files(path = system.file("extdata", "IMGT_data", "comparison",
                         "primer_sets", "IGH", package = "openPrimeR"),
                          pattern = "*\\.csv", full.names = TRUE)[1:3]
primer.data <- read_primers(primer.files)
# Read a mixture of FASTA/CSV files:
mixed.primers <- c(primer.fasta, primer.csv)
primer.data <- read_primers(mixed.primers)

# Select available settings
```

```
available.settings <- list.files(
     system.file("extdata", "settings", package = "openPrimeR"),
     pattern = "*.xml", full.names = TRUE)
# Select one of the settings and load them
filename <- available.settings[1]
settings <- read_settings(filename)
```

`insert_str` *String Insertion.*

Description

Inserts a string into another string at the speficied position.

Usage

```
insert_str(target, insert, index)
```

Arguments

`target`	The string to be modified.
`insert`	The string to be inserted.
`index`	The position where the insertion should take place.

Value

A string where `insert` is inserted into `target` at position `index`.

`interleave` *Interleave strings Combines the input vectors in an interleaved fashion.*

Description

Interleave strings Combines the input vectors in an interleaved fashion.

Usage

```
interleave(v1, v2)
```

Arguments

`v1`	Input string.
`v2`	Input string.

Value

The interleaved combination of `v1` and `v2`.

J.cvg *Template Coverage.*

Description

Determines the indices of covering primers for every template.

Usage

```
J.cvg(cvg.matrix)
```

Arguments

cvg.matrix	Binary matrix of covering events.

Value

A list with covering primers for every template.

joule.to.cal *Conversion from J to cal*

Description

Converts the input from Joule to calories.

Usage

```
joule.to.cal(val.J)
```

Arguments

val.J	Numeric Joule value.

Value

The value correspdoning to `val.J` in calories.

listToXml *List to XML*

Description

Convert List to XML.

Usage

```
listToXml(item, tag)
```

Arguments

item	
tag	xml tag

Details

Can convert list or other object to an xml object using xmlNode.

Value

xmlNode

Author(s)

David LeBauer, Carl Davidson, Rob Kooper

merge.ambig.primers *Merge similar primers*

Description

Merges similar primers contained in the input primer data frame.

Usage

```
## S3 method for class 'ambig.primers'
merge(primer.df, mode.directionality = c("fw", "rev", "both"), max.degeneracy)
```

Arguments

primer.df	Primer data frame.
mode.directionality	Analysis direction.
max.degeneracy	Maximal degeneracy of merged primers.

Value

A primer data frame where similar primers are merged into one entry.

`merge.binding.information` *Merge of Forward/Reverse Binding Information.*

Description

Determines binding events of individual and pairs of primers.

Usage

```
## S3 method for class 'binding.information'
merge(
  primers,
  fw.binding.filtered,
  rev.binding.filtered,
  mode.directionality = c("fw", "rev", "both"),
  idx.fw,
  idx.rev
)
```

Arguments

`primers`	The primer data frame.
`mode.directionality`	Primer directionality.
`idx.fw`	Index of fw primers.
`idx.rev`	Index of rev primers.
`fw.binding`	IRanges object with binding events of fw primers.
`rev.binding`	IRanges object with binding events of rev primers.

Value

IRanges with correct binding events.

merge.primer.entries *Merge similar primers*

Description

Merges the entries of similar entries in the input primer data frame, given a list with merge indices.

Usage

```
## S3 method for class 'primer.entries'
merge(opti.result, merge.idx, mode.directionality = c("fw", "rev", "both"))
```

Arguments

opti.result	Input primer data frame.
merge.idx	List of lists with merge indices (get.merge.idx).
mode.directionality	Direction of primers.

Value

A primer data frame where entries of similar primers are merged.

merge.primer.entries.single *Merge input sequences*

Description

Merges the input sequences given a list with merge indices.

Usage

```
## S3 method for class 'primer.entries.single'
merge(seqs, merge.idx)
```

Arguments

seqs	The input sequences.
merge.idx	List of list with merge indices.

Value

Merged input sequences according to the input merge indices.

`merge.select` *Select merge indices*

Description

Greedily identifies the smallest number of possible sequences merges that can be performed.

Usage

```
## S3 method for class 'select'
merge(merge.idx)
```

Arguments

`merge.idx`	list of lists containing the indices of possible merges

Value

The smallest number of possible merge operations as an index list.

`merge.template.decisions`
Merge Template Decisions.

Description

Merges the results for multiple template evaluations.

Usage

```
## S3 method for class 'template.decisions'
merge(eval.t)
```

Arguments

`eval.t`	List with evaluated template constraints

Value

List with merged boolean decisions.

mismatch.info *Information about Mismatches.*

Description

Computes information about mismatch binding events.

Usage

```
mismatch.info(primer, seqs)
```

Arguments

primer	Primer character vector.
seqs	Template binding sequences of primers as a XStringsView object.

Value

List with positions and number of mismatches of the primer in the seqs. The list contains the field mm.pos containing a list with the positions of the mismatches and the field Nbr containing a numeric vector with the number of mismatches per template binding event.

mismatch.mutation.check *Identification of Mutations Induced by Mismatch Binding Events.*

Description

Identifies whether mutations are induced by mismatch binding events.

Usage

```
mismatch.mutation.check(
  primer.df,
  template.df,
  mutation.types = c("stop_codon", "substitution")
)
```

Arguments

primer.df	A Primers object.
template.df	A Template object.
mutation.types	Character vector of the mutation types to be checked for.

Details

Checks for one primer and all covered templates whether any templates are bound with mismatches such that mismatches are induced. A numeric vector indicating which binding events induce a forbidden mismatch according to `mutation.types` is returned such that `1` indicates forbidden events and `0` allowed events.

Value

A list containing data frames where an entry of 1 is present if the `primer.seq` induces a mutation that is forbidden according to the provided `mutation.types`, otherwise 0.

---

mismatch.string.to.list *Conversion of Mismatch Postions String to List.*

Description

Conversion of Mismatch Postions String to List.

Usage

```
mismatch.string.to.list(mismatches)
```

Arguments

`mismatches`	A character vector where parenthesis give mismatches for a template binding event.

Value

A list with the mismatches for every template for every primer.

---

modify.col.rep *Modification of Column Names.*

Description

Modifies column names for frontend output.

Usage

```
modify.col.rep(template.df, for.shiny = TRUE)
```

Arguments

template.df	The data frame whose column names are to be modified.
for.shiny	Whether formatting should be for shiny.

Value

template.df with modified column names.

---

my.disambiguate *Disambiguation of Sequences.*

Description

Disambiguates the input sequences, but does not disambiguate highly generate sequences.

Usage

```
my.disambiguate(template.seqs, gap.char = "-", degen.cutoff = 2^10)
```

Arguments

template.seqs	A DNAStringSet object with sequences to disambiguate.
gap.char	The character indicating gaps in alignments.
degen.cutoff	The maximal degeneration of sequences to be disambiguated.

Value

A DNAStringSetList object with disambiguated sequences.

---

my.error *Custom Error*

Description

Creates an error with a custom class.

Usage

```
my.error(subclass, message, call = sys.call(-1), ...)
```

Arguments

subclass	String giving the specific type of error.
message	Message to be displayed to the user.
call	Environment where the error ocurred.
...	Other arguments to be passed to the condition function.

Value

Generates a custom error.

`my.read.fasta` *Read FASTA File.*

Description

Reads the input FASTA file.

Usage

```
my.read.fasta(fasta.file, NTs)
```

Arguments

`fasta.file`	The path to a FASTA file.
`NTs`	The allowed set of nucleotides.

Value

List with vectors of chars.

`my.warning` *Custom Warning.*

Description

Creates a warning with a custom class.

Usage

```
my.warning(subclass, message, call = sys.call(-1), ...)
```

Arguments

`subclass`	String giving the specific type of error.
`message`	Message to be displayed to the user.
`call`	Environment where the error ocurred.
`...`	Other arguments to the condition function.

Value

Generates a custom warning.

my_ggsave *Wrapper for the ggplot2::ggsave function.*

Description

Saves a plot using ggplot2's ggsave function.

Usage

```
my_ggsave(filename, plot = ggplot2::last_plot(), ...)
```

Arguments

filename	The filename to store the plot.
plot	The ggplot object.
...	Further arguments to the ggplot2 ggsave function.

Value

Stores p in fname.

my_rbind *Smartbind preserving classes.*

Description

Rbind allowing for column mismatch, retains the classes of the data frames. Motivation: smartbind/rbind.fill only keep the data.frame class but not additional classes.

Usage

```
my_rbind(...)
```

Arguments

...	Data frames.

Value

A data frame resulting from row binding of ....

nbr.of.repeats *Number of Repeats*

Description

Computes the number of dinucleotide repeats in the input sequences.

Usage

```
nbr.of.repeats(x)
```

Arguments

x	Input sequence strings.

Value

The maximal number of dinucleotide repeats in x.

nbr.of.runs *Number of Runs*

Description

Computes the longest run of a single character in the input sequence.

Usage

```
nbr.of.runs(x)
```

Arguments

x	Primer character sequences.

Value

The longest repeat of a single character in x.

opti *Getter for Optimization Constraints.*

Description

Gets the constraints on the physicochemical properties that are applied just before the optimization procedure using the `Input_Constraints` slot of the provided `DesignSettings` object `x`.

Usage

```
opti(x)

## S4 method for signature 'DesignSettings'
opti(x)
```

Arguments

`x`	A `DesignSettings` object.

Value

Gets the list of optimization constraints.

optiLimits *Getter for Optimization Constraint Limits.*

Description

Gets the limits for the constraints that are applied just before the optimization procedure using the `Input_Constraint_Boundaries` slot of the provided `DesignSettings` object `x`.

Usage

```
optiLimits(x)

## S4 method for signature 'DesignSettings'
optiLimits(x)
```

Arguments

`x`	A `DesignSettings` object.

Value

Gets the optimization constraint limits.

`optimize.ILP` *Solver for ILP Set Cover*

Description

Solves the primer set cover problem using an ILP formulation.

Usage

```
optimize.ILP(
  primer.df,
  template.df,
  settings,
  primer_conc,
  template_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  allowed.mismatches,
  allowed.other.binding.ratio,
  allowed.stop.codons,
  allowed.region.definition,
  disallowed.mismatch.pos,
  target.temps,
  required.cvg,
  fw.primers = NULL,
  diagnostic.location = NULL,
  timeout = Inf,
  updateProgress = NULL
)
```

Arguments

`primer.df`	Primer data frame to be optimized.
`template.df`	Template data frame with sequences.
`settings`	A `DesignSettings` object.
`primer_conc`	Primer concentration.
`template_conc`	Template concentration.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris ion concentration.
`allowed.mismatches`	The number of mismatches primers are allowed to have with the templates.

`allowed.other.binding.ratio`
: Ratio of primers allowed to bind to non-target regions.

`allowed.stop.codons`
: Consider mismatch binding events that induce stop codons.

`allowed.region.definition`
: Definition of the target binding sites used for evaluating the coverage. If `allowed.region.definition` is `within`, primers have to lie within the allowed binding region. If `allowed.region.definition` is `any`, primers have to overlap with the allowed binding region. The default is that primers have to bind within the target binding region.

`disallowed.mismatch.pos`
: The number of positions from the primer 3' end where mismatches should not be allowed. All primers binding templates with mismatches within `disallowed.mismatch.pos` from the 3' end are disregarded.

`target.temps`
: Target melting temperatures for primer sets in Celsius.

`required.cvg`
: Target coverage ratio of the templates by the primers.

`fw.primers`
: List with optimized primer data frames corresponding to `target.temps`. Only required for optimizing both strand directions and only in the second optimization run in order to check for cross dimerization.

`diagnostic.location`
: Directory for storing results.

`timeout`
: Timeout in seconds for the optimization with ILPs.

`updateProgress`
: Shiny progress callback function.

Value

List with optimization results.

`optimize.primer.cvg` *Greedy Optimization*

Description

Greedy approach for solving the primer set coverage problem.

Usage

```
optimize.primer.cvg(
  primers,
  template.df,
  mode.directionality,
  cur.opti.constraints,
  target.temp,
  allowed.mismatches,
  opti.limits,
  primer_conc,
```

```
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  updateProgress = NULL
)
```

Arguments

primers	Primer data frame to be optimized.
template.df	Template data frame.
mode.directionality	Primer direction.
cur.opti.constraints	List with optimization constraint settings.
target.temp	Target annealing temperature of the optimized primer set in Celsius.
allowed.mismatches	The number of mismatches primers are allowed to have with the templates.
opti.limits	List with optimization limits.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris ion concentration.
updateProgress	Shiny progress callback function.
template_conc	Template concentration.

Value

List with optimization data.

---

optimize.template.binding.regions.dir

Optimization of Binding Regions

Description

Optimizes the template binding regions.

Usage

```
optimize.template.binding.regions.dir(
  template.df,
  annealing.temperature = NULL,
  primer.lengths,
  mode.directionality = c("fw", "rev", "both")
)
```

Arguments

`template.df`	Template data frame.
`annealing.temperature`	Temperature at which to compute secondary structures.
`primer.lengths`	Target length of primers that are to be used.
`mode.directionality`	Direction of primers.

Value

List with intervals indicating improved primer binding regions.

`optimize.template.binding.regions.single` *Optimization of Template Binding*

Description

Optimizes template binding regions according to secondary structures.

Usage

```
optimize.template.binding.regions.single(
  template.df,
  annealing.temperature,
  primer.lengths,
  mode.directionality = c("fw", "rev")
)
```

Arguments

`template.df`	Template data frame.
`annealing.temperature`	Temperature at which to compute secondary structures.
`primer.lengths`	Target length of primers that are to be used.
`mode.directionality`	Direction of primers.

Value

List with new binding intervals for every template.

Output *Output Functionalities.*

Description

`write_primers` Writes a set of primers to disk, either as a FASTA or CSV file.

`write_settings` Stores primer analysis settings to a file in XML format.

`write_templates` Stores a set of templates as a FASTA or CSV file.

`create_report` Creates a PDF report for analyzed primer sets.

`create_coverage_xls` Creation of an XLS spreadsheet providing an overview of the covered template sequences for each primer. Each cell in the spreadsheet indicates a coverage event between a primer and template using color codes. Identified coverage events are indicated by green, while primer-template pairs without coverage are indicated by red. In case that a primer binding condition (see `CoverageConstraints`) was active when computing the coverage, the numeric value of the coverage condition is annotated for each cell.

Usage

```
write_templates(template.df, fname, ftype = c("FASTA", "CSV"))

write_primers(primer.df, fname, ftype = c("FASTA", "CSV"))

create_coverage_xls(primer.df, template.df, fname, settings)

create_report(
  primers,
  templates,
  fname,
  settings,
  sample.name = NULL,
  used.settings = NULL,
  ...
)

write_settings(settings, fname)
```

Arguments

`template.df`	An object of class `Templates`.
`fname`	The path to the output file.
`ftype`	A character vector giving the type of the file. This can either be "FASTA" or "CSV" (default: "FASTA").
`primer.df`	An object of class `Primers`.
`settings`	A `DesignSettings` object to be stored to disk.

primers	To create a report for a single primer set, please provide an evaluated Primers object. For creating a report comparing multiple primer sets, please provide a list of Primers objects.
templates	If primers is a Primers object, templates should be a Templates object. If primers is a list of Primers objects, templates should be a list of Templates objects of the same length as primers.
sample.name	An identifier for your analysis. By default (NULL), the sample identifier is selected from the Run column of the input templates.
used.settings	A named list (with fields fw and rev) containing the relaxed settings for designing forward/reverse primers. By default (NULL), the relaxed settings are not shown in the report.
...	required.cvg (optional, default: 1), the desired coverage ratio if primers is a single primer set.

Value

write_templates stores templates to fname.

write_primers stores primers to disk.

create_coverage_xls stores information on the primer coverage in a spreadsheet.

create_report Creates a PDF file summarizing the results from analyzing one or multiple sets of primers.

write_settings returns the status from closing the connection to the output file.

Note

Creating the report requires the external programs Pandoc (http://pandoc.org) and LaTeX (http://latex-project.org).

Examples

```
data(Ippolito)
# Store templates as FASTA
fname.fasta <- tempfile("my_templates", fileext = ".fasta")
write_templates(template.df, fname.fasta)
# Store templates as CSV
fname.csv <- tempfile("my_templates", fileext = ".csv")
write_templates(template.df, fname.csv, "CSV")

data(Ippolito)
# Store primers as FASTA
fname.fasta <- tempfile("my_primers", fileext = ".fasta")
write_primers(primer.df, fname.fasta)
# Store primers as CSV
fname.csv <- tempfile("my_primers", fileext = ".csv")
write_primers(primer.df, fname.csv, "CSV")

data(Ippolito)
filename <- tempfile("cvg_overview", fileext = ".xls")
```

```
# Store coverage of a single primer in an XLS file:
my.primers <- primer.df[3,]
cvd <- unique(unlist(strsplit(my.primers$Covered_Seqs, split = ",")))
m <- match(cvd, template.df$Identifier)
my.templates <- template.df[m,]
create_coverage_xls(my.primers, my.templates, filename, settings)

setting.xml <- system.file("extdata", "settings",
                "C_Taq_PCR_high_stringency.xml", package = "openPrimeR")
settings <- read_settings(setting.xml)
# Creation of a report for a single primer set
data(Ippolito)
out.file.single <- tempfile("evaluation_report", fileext = ".pdf")
create_report(primer.df, template.df, out.file.single, settings)
# Creation of a report for multiple primer sets
data(Comparison)
set.sizes <- sapply(primer.data, nrow)
sel.sets <- order(set.sizes)[1:2]
out.file.comp <- tempfile("comparison_report", fileext = ".pdf")
create_report(primer.data[sel.sets], template.data[sel.sets], out.file.comp, settings)

# Store settings to disk
xml <- system.file("extdata", "settings",
       "C_Taq_PCR_high_stringency.xml", package = "openPrimeR")
settings <- read_settings(xml)
out.file <- tempfile("my_settings", fileext = ".xml")
write_settings(settings, out.file)
```

`pair_primers` *Pairing of Forward and Reverse Primers.*

Description

Pairs forward and reverse primers such that coverage is maximized for every pair.

Usage

```
pair_primers(primer.df, template.df)
```

Arguments

`primer.df`	An object of class `Primers`.

Value

An object of class `Primers` containing the paired primers.

parse.constraints *Parse XML Constraint Data.*

Description

Parses the constraint settings contained in an XML object.

Usage

```
parse.constraints(xml_data)
```

Arguments

xml_data	XML object from a parsed XML file.

Value

List with constraint settings.

parse.header *Parse FASTA headers*

Description

Parses the headers of a FASTA file.

Usage

```
parse.header(hdr, delim, hdr.str, id.column)
```

Arguments

hdr	The headers (> entries) of a FASTA File.
delim	The delimiter used to separate distinct fields in the headers. For example, | for headers such as > E.coli | GeneX | ...
hdr.str	Names of the fields appearing in the header.
id.column	Field in the header to be used used as an identifier for the sequences.

Value

Data frame with structured information from the headers.

parse.IMGT.gene.groups *Parser for IMGT Groups.*

Description

Parses IMGT group information contained in FASTA headers.

Usage

```
parse.IMGT.gene.groups(IDs)
```

Arguments

IDs	Group information strings to be parsed.

Value

Data frame with structured group information.

parse.oligo.results *Parser for OligoArrayAux Dimerization Data.*

Description

Parses the free energies and structures of OligoArrayAux.

Usage

```
parse.oligo.results(deltaG.file, struct.file)
```

Arguments

deltaG.file	A path to a file with OligoArrayAux energies.
struct.file	A path to a file with OligoArrayAux structures.

Value

A data frame with structures and free energies.

plot.all.cvg.info *Plots Coverage Information*

Description

Visualizes all coverage-related data.

Usage

```
## S3 method for class 'all.cvg.info'
plot(
  sample,
  results.loc,
  primers,
  template.df,
  mode.directionality,
  identifier = c("filtering", "optimized"),
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  settings,
  required.cvg,
  used.settings = NULL
)
```

Arguments

sample	Primer design run identifier.
results.loc	Location where the filtering results are stored.
primers	Primer data frame.
template.df	Template data frame.
mode.directionality	Design direction.
identifier	Identifies whether filtering or optimization info should be displayed.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris ion concentration.
settings	The DesignSettings object.
required.cvg	The required coverage.
used.settings	A list containing DesignSettings objects for the 'fw' and 'rev' optimization.

Value

Writes plots to files.

`plot.all.filtering.stats` *Plot Filtering Stats.*

Description

Plots filtering statistics.

Usage

```
## S3 method for class 'all.filtering.stats'
plot(
  results.loc,
  sample,
  excluded.df,
  filtered.stats,
  stats.relax,
  template.df
)
```

Arguments

`results.loc`	Location where the filtering results are stored.
`sample`	Primer design run identifier.
`excluded.df`	Data frame with excluded primers.
`filtered.stats`	Filtering statistics data frame.
`stats.relax`	Filtering statistics after relaxation.
`template.df`	Template data frame.

Value

Write-out of filtering results.

plot.Delta.DeltaG *Delta DeltaG Plot*

Description

Plots the difference between the free energy of constrained and unconstrained foldings.

Usage

```
## S3 method for class 'Delta.DeltaG'
plot(constrained.foldings, stratify = FALSE)
```

Arguments

constrained.foldings	Data frame with info from constrained foldings.
stratify	Stratify according to template groups?

Value

Plot of Delta DeltaG.

plot.dimer.dist *Plot Dimer DeltaG*

Description

Plot the distribution of dimerization free energies.

Usage

```
## S3 method for class 'dimer.dist'
plot(dimer.data, deltaG.cutoff)
```

Arguments

dimer.data	Data frame with dimerization information.
deltaG.cutoff	Free energy cutoff for dimerization.

Value

A plot of dimerization free energies.

`plot.excluded.hist` *Plot of Excluded Primers*

Description

Plots histogram of excluded primers.

Usage

```
## S3 method for class 'excluded.hist'
plot(excluded.df, filtered.stats, template.df)
```

Arguments

`excluded.df`	Data frame with excluded primers.
`filtered.stats`	Data frame with statistics of the filtering procedure.
`template.df`	Template data frame.

Value

A plot of excluded primers.

`plot.filtering.runtime`
Plot Filtering Runtimes

Description

Plots the runtimes of individual evaluation steps in the filtering procedure.

Usage

```
## S3 method for class 'filtering.runtime'
plot(filtered.stats)
```

Arguments

`filered.stats`	Stats from filtering.

Value

A plot of the runtime for each filtering step.

plot.filtering.stats *Plot of Overall Filtering Stats.*

Description

Plots the number of primers remaining after each filtering step.

Usage

```
## S3 method for class 'filtering.stats'
plot(stats, stats.relax = NULL)
```

Arguments

stats	Statistics on the filtering procedure
stats.relax	Statistic on the filtering procedure after relaxation.

Value

A plot for the number of primers after filtering.

plot.filtering.stats.cvg *Plot of Filtering Stats for Coverage.*

Description

Plots the remaining coverage after each filtering step.

Usage

```
## S3 method for class 'filtering.stats.cvg'
plot(stats, stats.relax = NULL)
```

Arguments

stats	Statistics of the filtering procedure.
stats.relax	Statistic of the relaxation procedure.

Value

A plot showing the possible coverage after each filtering step.

Plots *Plotting Functions.*

Description

`plot_cvg_vs_set_size` Plots the coverage ratios of the input primer sets against the size of the sets.

`plot_penalty_vs_set_size` Plots the penalties of the input primer sets against the number of primers contained in each set. The penalties are computed using `score_primers` where more information is provided on how to set `alpha`.

`plot_primer_subsets` Visualizes the coverage of optimized primer subsets.

`plot_primer` Visualizes the binding positions of every primer relative to the target binding region in the corresponding template sequences.

`plot_template_cvg` Creates a bar plot visualizing the covered templates.

`plot_primer_cvg` Shows which groups of templates are covered by individual primers.

`plot_constraint` Shows the distribution of the primer properties. The current constraint settings are indicated with dashed lines.

`plot_constraint_fulfillment` Visualizes which primers pass the constraints and which primers break the constraints

`plot_cvg_constraints` Plots the distribution of the coverage constraint values.

`plot_constraint_deviation` Plots the deviation of primer properties from the target ranges.

`plot_primer_binding_regions` Visualizes the number of binding events of the primers with respect to the allowed binding regions in the templates.

`plot_conservation` Plots the template sequence conservation (range [0,1]) according to the Shannon entropy of the sequences.

Usage

```
plot_conservation(entropy.df, alignments, template.df, gap.char = "-")

plot_primer_binding_regions(
  primers,
  templates,
  direction = c("both", "fw", "rev"),
  group = NULL,
  relation = c("fw", "rev"),
  region.names = c("Binding region", "Amplification region"),
  ...
)

plot_constraint(
  primers,
  settings,
```

```
  active.constraints = names(constraints(settings)),
  ...
)

plot_constraint_fulfillment(
  primers,
  settings,
  active.constraints = names(constraints(settings)),
  plot.p.vals = FALSE,
  ...
)

plot_cvg_constraints(
  primers,
  settings,
  active.constraints = names(cvg_constraints(settings)),
  ...
)

plot_constraint_deviation(
  primers,
  settings,
  active.constraints = names(constraints(settings)),
  ...
)

plot_cvg_vs_set_size(
  primer.data,
  template.data,
  show.labels = TRUE,
  highlight.set = NULL
)

plot_penalty_vs_set_size(
  primer.data,
  settings,
  active.constraints = names(constraints(settings)),
  alpha = 0
)

plot_primer_subsets(primer.subsets, template.df, required.cvg = 1)

plot_primer(
  primer.df,
  template.df,
  identifier = NULL,
  relation = c("fw", "rev"),
  region.names = c("Binding region", "Amplification region")
```

```
)

plot_template_cvg(primers, templates, per.mismatch = FALSE, ...)

plot_primer_cvg(primers, templates, per.mismatch = FALSE, ...)
```

Arguments

entropy.df	A data frame with entropies. Each row gives the entropies of a group of related template sequences for all columns of the alignment.
alignments	A list with DNABin alignment objects corresponding to the groups (rows) in the alignment.
template.df	An object of class Templates containing the template sequences.
gap.char	The gap char in the alignments. By default, gap.char is set to "-".
primers	Either a single Primers object with evaluated primer coverage or a list containing such Primers objects.
templates	If primers is a Primers object, templates should be a Templates object. If primers is a list, then templates should be a list of Templates objects.
direction	The directionality of primers to be plotted. This can either be "both" to plot primers of both directions (the default), "fw" to plot only forward primers, or "rev" to plot only reverse primers.
group	Optional identifiers of template groups for which binding events should be determined. By default, group is set to NULL such that all templates are considered.
relation	Whether binding positions are computed relative to forward ("fw") or reverse ("rev") binding regions. The default is "fw".
region.names	Character vector of length 2 providing the names of the binding and amplification region.
...	Optional arguments groups (a character vector of groups to be plotted when primers is a single primer set), highlight.set (the identifier of a primer set to be highlighted when primers is a list), ncol (a numeric indicating the number of facet columns if primers is a list), deviation.per.primer (a boolean indicating whether constraint deviations should be plotted per primer rather than per constraint if primers is a list)
settings	An object of class DesignSettings containing the constraints to be considered.
active.constraints	A character vector containing the identifiers to be considered for plotting. By default, active.constraints is NULL such that all computed constraints found in settings are plotted.
plot.p.vals	An optional logical argument indicating whether p-values computed via primer_significance should be annotated in the plot. The default is FALSE.
primer.data	List with objects of class Primers containing the primer sets that are to be compared.
template.data	List with objects of class Templates containing the templates corresponding to primer.data.

show.labels	Whether the identifiers of the primer sets should be annotated in the plot. The default is TRUE.
highlight.set	A character vector providing the identifiers of primer sets to highlight. By default, highlight.set is NULL such that no highlighting takes place.
alpha	A numeric in the range [0,1] defining the trade-off between the maximal deviation of a constraint (large alpha) and all constraint deviations (large alpha). By default, alpha is set to 0 such that the absolute deviation across all constraints is considered.
primer.subsets	A list with optimal primer subsets, each of class Primers. The k-th list entry should correspond to an object of class Primers representing the primer subset of size k whose coverage ratio is the largest among all possible subsets of size k.
required.cvg	The required coverage ratio. The default is 100%; this value is plotted as a horizontal line.
primer.df	An object of class Primers containing primers with evaluated primer coverage.
identifier	Identifiers of primers that are to be considered. If identifier is set to NULL (the default), all primers are considered.
per.mismatch	A logical specifying whether the visualization should be stratified according to the allowed number of mismatches. By default, per.mismatch is set to FALSE such that the overall coverage is plotted.

Details

The deviations for plot_constraint_deviation are computed in the following way. Let the minimum and maximum allowed constraint values be given by the interval $[s, e]$ and the observed value be p. Then, if $p < s$, we output $-p/|s|$, if $p > e$ we output $p/|e|$, and otherwise, i.e. if $s <= p <= e$, we output 0.

The primer.subsets argument for plot_primer_subsets can be computed using subset_primer_set. The line plot indicates the ratio of covered templates when considering all primers in a primer set of a given size. The bar plots indicate the coverage ratios of individual primers in a set. The target coverage ratio is indicated by a horizontal line. Bars exceeding the target ratio possibly indicate the existence of redundant coverage events.

Value

plot_conseration returns a plot showing the degree of sequence conservation in the templates.

plot_primer_binding_regions returns a plot of the primer binding regions.

plot_constraint returns a plot showing the distribution of primer properties.

plot_constraint_fulfillment returns a plot indicating the constraints that are fulfilled by the input primers.

plot_cvg_constraints returns a plot showing the distribution of the coverage constraint values.

plot_constraint_deviation returns a plot showing the deviations of the primer properties from the target constraints.

plot_cvg_vs_set_size returns a plot of coverage vs set size.

plot_penalty_vs_set_size returns a plot of constraint penalties vs primer set sizes.

plot_primer_subsets plots the coverages of the primer subsets provided via primer.subsets.

plot_primer plots the primer binding sites in the templates.

plot_template_cvg creates a plot showing the number of covered template sequences.

plot_primer_cvg creates a plot showing the coverage of individual primers.

Note

Computing the conservation scores for using plot_conservation requires the MAFFT software for multiple alignments (http://mafft.cbrc.jp/alignment/software/).

Examples

```
data(Ippolito)
# Select binding regions for every group of templates and plot:
template.df <- select_regions_by_conservation(template.df, win.len = 30)
if (length(template.df) != 0) {
     p1 <- plot_conservation(attr(template.df, "entropies"),
                             attr(template.df, "alignments"), template.df)
}
# Select binding regions for all templates and plot:
data(Ippolito)
template.df <- select_regions_by_conservation(template.df, by.group = FALSE)
if (length(template.df) != 0) {
     p2 <- plot_conservation(attr(template.df, "entropies"),
                             attr(template.df, "alignments"), template.df)
}

# Primer binding regions of a single primer set
data(Ippolito)
p <- plot_primer_binding_regions(primer.df, template.df)
# Primer binding regions of multiple primer sets
data(Comparison)
p.comp <- plot_primer_binding_regions(primer.data[1:3], template.data[1:3])

# Plot histogram of constraints for a single primer set
data(Ippolito)
p <- plot_constraint(primer.df, settings,
                     active.constraints = c("gc_clamp", "gc_ratio"))
# Compare constraints across multiple primer sets
data(Comparison)
p.cmp <- plot_constraint(primer.data[1:3], settings,
                         active.constraints = c("gc_clamp", "gc_ratio"))

# Plot fulfillment for a single primer set:
data(Ippolito)
p <- plot_constraint_fulfillment(primer.df, settings)
# Plot fulfillment for multiple primer sets:
data(Comparison)
p.cmp <- plot_constraint_fulfillment(primer.data[1:5], settings)
```

```
# Plot coverage constraints of a single primer set
data(Ippolito)
p <- plot_cvg_constraints(primer.df, settings)
# Plot coverage constraints for mulitple primer sets
data(Comparison)
p.cmp <- plot_cvg_constraints(primer.data[1:2], settings)

# Deviations for a single primer set
data(Ippolito)
p.dev <- plot_constraint_deviation(primer.df, settings)
# Deviations for multiple primer sets
data(Comparison)
p.dev.cmp <- plot_constraint_deviation(primer.data, settings)

# Plot coverage vs primer set size
data(Comparison)
p <- plot_cvg_vs_set_size(primer.data, template.data)

# Plot penalties vs number of primers
data(Comparison)
p <- plot_penalty_vs_set_size(primer.data, settings)

# Plot the coverage of optimal primer subsets
data(Ippolito)
primer.subsets <- subset_primer_set(primer.df, template.df, k = 3)
p <- plot_primer_subsets(primer.subsets, template.df)

# Plot of individual primer binding positions
data(Ippolito)
p <- plot_primer(primer.df[1,], template.df[1:30,])

# Visualize the template coverage of a single primer set
data(Ippolito)
p.cvg <- plot_template_cvg(primer.df, template.df)
# Stratify by allowed mismatches:
p.mm.cvg <- plot_template_cvg(primer.df, template.df, per.mismatch = TRUE)
# Compare the coverage of multiple primer sets
data(Comparison)
p.cmp.cvg <- plot_template_cvg(primer.data[1:2], template.data[1:2])
# Stratify by allowed mismatches:
p.cmp.cvg.mm <- plot_template_cvg(primer.data[1:2], template.data[1:2],
                                  per.mismatch = TRUE)

# Plot expected coverage per primer
data(Ippolito)
p.cvg <- plot_primer_cvg(primer.df, template.df)
# Plot coverage stratified by allowed mismatches:
p.cvg.mm <- plot_primer_cvg(primer.df, template.df, per.mismatch = TRUE)
# Plot coverage of multiple primer sets
data(Comparison)
p.cvg.cmp <- plot_primer_cvg(primer.data[1:3], template.data[1:3])
```

plot_constraint,list-method

Boxplot for Comparing Constraints.

Description

Creates a boxplot visualizing the physicochemical properties of multiple primer sets.

Usage

```
## S4 method for signature 'list'
plot_constraint(
  primers,
  settings,
  active.constraints,
  highlight.set = NULL,
  nfacets = NULL
)
```

Arguments

primers	List with evaluated objects of class Primers. Each list element corresponds to a single primer set.
settings	A DesignSettings object containing the constraints to be plotted.
active.constraints	The names of the constraints to be plotted.
highlight.set	Identifiers of primer sets to be highlighted.
nfacets	A numeric providing the number of facet columns to show. By default nfacets is NULL such that the number of facet columns is chosen automatically.
constraint.settings	List with settings for each constraint.

Value

Boxplot comparing the values of the properties specified by constraints.

plot_constraint,Primers-method

Histogram of Constraint Values.

Description

Plots a histogram of constraint values.

Usage

```
## S4 method for signature 'Primers'
plot_constraint(primers, settings, active.constraints)
```

Arguments

primers	An evaluated object of class Primers.
settings	A DesignSettings object containing the settings for the constraints to be plotted.
active.constraints	Identifiers of constraints to be plotted. provided settings are used to visualize the desired ranges of constraints. If active.constraints is not provided, the plotting method will automatically try to plot all constraints defined in settings.

Value

A histogram of constraint values for the properties specified by constraints.

---

plot_constraint.histogram

Histogram of Constraints.

Description

Plots a histogram of constraint values.

Usage

```
plot_constraint.histogram(
  primer.df,
  con.cols,
  con.identifier,
  boundaries = NULL,
  x.limits = NULL
)
```

Arguments

primer.df	Primer data frame, not necessarily a Primers object.
con.cols	Constraint identifiers in primer.df to plot.
con.identifier	Name of the constraint to plot.
boundaries	List with constraint settings.
x.limits	Interval limiting the extent of the x-axis.

Value

A constraint histogram plot.

`plot_constraint.histogram.nbr.mismatches`
Histogram of Number of Mismatches.

Description

Plots a histogram of mismatches.

Usage

```
plot_constraint.histogram.nbr.mismatches(primer.df, allowed.mismatches)
```

Arguments

`primer.df`	Primer data frame.
`allowed.mismatches`	Number of allowed mismatches.

Value

A plot of the number of primer mismatches.

`plot_constraint.histogram.primer.efficiencies`
Histogram of Efficiencies

Description

Plots a histogram of primer efficiencies.

Usage

```
plot_constraint.histogram.primer.efficiencies(primer.df, opti.constraints)
```

Arguments

`primer.df`	Primer data frame.
`opti.constraints`	List with constraint settings.

Value

A plot of primer efficiencies.

plot_constraint_deviation,list-method

Plot of Constraint Deviations for Multiple Primer Sets.

Description

Plots a box plot of the absolute mean deviation of each primer for comparing multiple primer sets.

Usage

```
## S4 method for signature 'list'
plot_constraint_deviation(
  primers,
  settings,
  active.constraints,
  deviation.per.primer = FALSE
)
```

Arguments

settings	A DesignSettings object containing the target ranges for the primer properties.
active.constraints	Constraint identifiers to be plotted.
deviation.per.primer	Whether to show the deviation per primer or per constraint.
constraint.df	An evaluated object of class Primers.

Value

A boxplot of deviations

plot_constraint_deviation,Primers-method

Plot of Constraint Deviations for a Single Primer Set.

Description

Plots a box plot of deviations of primer properties from the target ranges.

Usage

```
## S4 method for signature 'Primers'
plot_constraint_deviation(primers, settings, active.constraints)
```

Arguments

primers	An evaluated object of class Primers.
settings	A DesignSettings object containing the target ranges for the primer properties.
active.constraints	Constraint identifiers to be plotted.

Value

A boxplot of deviations

---

plot_constraint_fulfillment,list-method

Comparison of Evaluation Results.

Description

Plots the percentage of primers fulfilling the specified constraints for multiple primer sets.

Usage

```
## S4 method for signature 'list'
plot_constraint_fulfillment(
  primers,
  settings,
  active.constraints,
  plot.p.vals = FALSE,
  ncol = 2,
  highlight.set = NULL
)
```

Arguments

primers	A list of Primers objects.
settings	A DesignSettings object.
active.constraints	The identifiers of constarints to be plotted for fulfillment.
plot.p.vals	Whether p-values from Fisher's exact test should be annotated for every primer set.
ncol	The number of columns for facet wrap.
highlight.set	Identifiers of primer sets to be highlighted.

Value

Plot indicating the ratio of primers fulfilling the constraints specified in constraint.settings for each primer set in primers.

plot_constraint_fulfillment,Primers-method

Overview of Constraint Fulfillment.

Description

Plots an overview of which primers passed the filtering constraints and which primers did not.

Usage

```
## S4 method for signature 'Primers'
plot_constraint_fulfillment(
  primers,
  settings,
  active.constraints,
  plot.p.vals = TRUE
)
```

Arguments

primers	A Primers object.
settings	A DesignSettings object.
active.constraints	The identifiers of constarints to be plotted for fulfillment.
plot.p.vals	Show p-value from Fisher's exact test for the significance of primer constraint fulfillment in comparison to reference primer sets.

Value

A data frame with statistics on fulfilled constraints.

plot_cvg_constraints,list-method

Plot for Comparing Primer Coverage Constraints.

Description

Plot for Comparing Primer Coverage Constraints.

Usage

```
## S4 method for signature 'list'
plot_cvg_constraints(
  primers,
  settings,
  active.constraints,
  highlight.set = NULL
)
```

Arguments

primers	List with objects of class Primers.
settings	A DesignSettings object.
active.constraints	Names of the coverage constraints to be plotted.
highlight.set	Primer sets to highlight in the plot.

Value

Plot of primer coverage constraints for multiple sets.

---

plot_cvg_constraints,Primers-method

Histogram of Coverage Constraints.

Description

Plots a histogram of coverage constraint values.

Usage

```
## S4 method for signature 'Primers'
plot_cvg_constraints(primers, settings, active.constraints)
```

Arguments

primers	A Primers object.
settings	A DesignSettings object.
active.constraints	Names of coverage constraints to be plotted.

Value

A plot of coverage constraints.

`plot_primer.comparison.box` *Boxplot for Primer Comparison*

Description

Constructs a box plot showing constraint values for each primer set.

Usage

```
plot_primer.comparison.box(
  primer.data,
  con.identifier,
  con.cols,
  boundaries,
  y.limits = NULL,
  show.points = TRUE,
  highlight.set = NULL,
  nfacets = NULL
)
```

Arguments

`primer.data`	List with primer data frames.
`con.identifier`	Identifier of constraint to be plotted.
`con.cols`	Column names with the constraint values in the primer data frames.
`boundaries`	List with constraint settings.
`y.limits`	Limits for the extent of the y-axis.
`show.points`	If `TRUE` (the default), individual data points are visualized in the boxplot, otherwise they are not shown.
`highlight.set`	The identifier of a primer set to highlight in the plot.
`nfacets`	A numeric providing the number of facet columns to show. By default `nfacets` is `NULL` such that the number of facet columns is chosen automatically.

Value

A boxplot for primer comparison.

plot_primer.comparison.mismatches *Plot Primer Mismatches*

Description

Plots primer mismatches for every set.

Usage

```
plot_primer.comparison.mismatches(
  primer.data,
  template.data,
  allowed.mismatches,
  highlight.set = NULL
)
```

Arguments

primer.data	List with primer data frames.
template.data	List with template data frames.
allowed.mismatches	Allowed mismatches.
highlight.set	Primer sets to highlight in the plot.

Value

Plot of mismatches for comparison.

plot_primer_binding_regions,list,list-method *Plot of Primer Binding Regions for Multiple Sets.*

Description

Plots the primer binding regions for every primer set.

Usage

```
## S4 method for signature 'list,list'
plot_primer_binding_regions(
  primers,
  templates,
  direction = c("both", "fw", "rev"),
  group = NULL,
```

```
  relation = c("fw", "rev"),
  region.names = c("Binding region", "Amplification region"),
  highlight.set = NULL
)
```

Arguments

primers	List with primer data frames.
templates	List with template data frames.
direction	Direction of primers.
group	Template groups to plot. This defaults to plotting all groups.
relation	Plot binding region relative to forward binding region or reverse?
region.names	Names for the primer binding region and the amplified region.
highlight.set	Primer sets to highlight in the plot.

Value

A plot for primer binding region comparison.

---

plot_primer_binding_regions,Primers,Templates-method

Plot of Primer Binding Regions for a Single Primer Set.

Description

Plots the primer binding regions in the templates.

Usage

```
## S4 method for signature 'Primers,Templates'
plot_primer_binding_regions(
  primers,
  templates,
  direction = c("both", "fw", "rev"),
  group = NULL,
  relation = c("fw", "rev"),
  region.names = c("Binding region", "Amplification region")
)
```

Arguments

primers	An object of class `Primers` with annotated primer coverage.
templates	An object of class `Templates` providing the template sequences corresponding to `primers`.
direction	Primer direction

group	The template groups for which binding events should be determined. By default, group is set to NULL such that all templates are considered.
relation	A character vector specifying whether binding region data shall be plotted relative to the forward (fw) or reverse (rev) target binding regions.
region.names	Names for the primer binding region and the amplified region.

Value

A histogram of primer binding regions.

`plot_primer_cvg,list,list-method`
Plot Multiple Primer Coverages.

Description

Plots the coverage of individual primers for multiple sets.

Usage

```
## S4 method for signature 'list,list'
plot_primer_cvg(primers, templates, per.mismatch = FALSE)
```

Arguments

primers	List with Primers objects.
templates	List with Templates objects.
per.mismatch	Whether the coverage should be broken down for individual settings of allowed mismatches.

Value

A bar plot showing the coverage of individual primers.

plot_primer_cvg,Primers,Templates-method

Plot Individual Primer Coverage.

Description

Shows which templates are covered by individual primers.

Usage

```
## S4 method for signature 'Primers,Templates'
plot_primer_cvg(primers, templates, per.mismatch = FALSE, groups = NULL)
```

Arguments

per.mismatch	Whether the coverage should be broken down for individual settings of allowed mismatches.
p.df	Primer data frame.
template.df	Template data frame.
excluded.seqs	Identifiers of templates that should not be considered.

Value

A bar plot showing the coverage of individual primers.

plot_primer_cvg_mismatches

Plot of Individual Primer Coverage and Mismatches.

Description

Plots the coverage of individual primers for different mismatch settings.

Usage

```
plot_primer_cvg_mismatches(
  primer.df,
  template.df,
  groups = NULL,
  nfacets = NULL
)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.
groups	Optional identifiers of template groups to be considered. If not provided, all template groups are considered.
nfacets	A numeric providing the number of facet columns to use. By default, nfacets is set to NULL such that a suitable number of columns is chosen automatically.

Value

A bar plot showing the coverage of individual primers for different mismatch settings.

---

plot_primer_cvg_unstratified

Plot Individual Primer Coverage.

Description

Plots the coverage of individual primers.

Usage

```
plot_primer_cvg_unstratified(p.df, template.df, groups = NULL)
```

Arguments

p.df	Primer data frame.
template.df	Template data frame.
groups	Optional identifiers of template groups to be considered. If not provided, all template groups are considered.

Value

A bar plot showing the coverage of individual primers.

`plot_template_cvg,list,list-method`
Templates Coverage for Multiple Primer Sets.

Description

Plots the coverage of multiple primer sets.

Usage

```
## S4 method for signature 'list,list'
plot_template_cvg(primers, templates, per.mismatch, highlight.set = NULL)
```

Arguments

`primers`	List with primer data frames.
`templates`	List with template data frames.
`highlight.set`	Primer sets to be highlighted.
`colors`	Color for every primer set.

Value

A plot for comparing primer coverage.

`plot_template_cvg,Primers,Templates-method`
Bar Plot of Template Coverage.

Description

Creates a bar plot showing the coverage for every group of template sequences.

Usage

```
## S4 method for signature 'Primers,Templates'
plot_template_cvg(primers, templates, per.mismatch, groups = NULL)
```

Arguments

`primers`	A `Primers` object with evaluated primer coverage.
`templates`	A `Templates` object containing the template sequences.
`per.mismatch`	Whether to stratify by mismatches.
`groups`	Identifiers of template groups for which plot should be created. By default, `groups` is set to `NULL` such that all templates are considered. according to the number of mismatches between primer-template pairs.

Value

A plot showing the number of covered template sequences.

`plot_template_cvg_comparison_mismatch`
Templates Coverage for Multiple Primer Sets.

Description

Plots the coverage of multiple primer sets.

Usage

```
plot_template_cvg_comparison_mismatch(primers, templates, highlight.set = NULL)
```

Arguments

`primers`	List with primer data frames.
`templates`	List with template data frames.
`highlight.set`	Primer sets to be highlighted.

Value

A plot for comparing primer coverage.

`plot_template_cvg_comparison_unstratified`
Templates Coverage for Multiple Primer Sets.

Description

Plots the coverage of multiple primer sets.

Usage

```
plot_template_cvg_comparison_unstratified(
  primers,
  templates,
  highlight.set = NULL
)
```

Arguments

`primers`	List with primer data frames.
`templates`	List with template data frames.
`highlight.set`	Primer sets to be highlighted.

Value

A plot for comparing primer coverage.

`plot_template_cvg_mismatches`
Bar Plot of Template Coverage for Mismatches.

Description

Creates a bar plot showing the coverage for every group of template sequences.

Usage

```
plot_template_cvg_mismatches(
  primer.df,
  template.df,
  groups = NULL,
  nfacets = 2
)
```

Arguments

`primer.df`	A `Primers` object.
`template.df`	A `Templates` object.
`groups`	Identifiers of template groups for which plot should be created. By default, `groups` is set to `NULL` such that all templates are considered.
`nfacets`	The number of facets columns to plot. By default, `nfacets` is set to 2.

Value

A plot showing the number of covered template sequences.

`plot_template_cvg_unstratified`
Bar Plot of Template Coverage.

Description

Creates a bar plot showing the coverage for every group of template sequences.

Usage

```
plot_template_cvg_unstratified(primers, templates, groups = NULL)
```

Arguments

primers	A Primers object with evaluated primer coverage.
templates	A Templates object containing the template sequences.
groups	Identifiers of template groups for which plot should be created. By default, groups is set to NULL such that all templates are considered. according to the number of mismatches between primer-template pairs.

Value

A plot showing the number of covered template sequences.

---

plot_template_structure

Plot of Template Folding Energies.

Description

Plots the DeltaDeltaG of template folding, which is the difference between the free energy change of the unconstrained folding and the free energy change of the constrained folding.

Usage

```
plot_template_structure(fold.df)
```

Arguments

fold.df	A data frame with free energies for the template regions.

Value

A plot of DeltaDeltaG.

---

pos.to.range

Conversion of Positions to Ranges.

Description

Converts two numeric values to a range.

Usage

```
pos.to.range(pos1, pos2)
```

Arguments

pos1	The first value.
pos2	The second value.

Value

A character vector range.

---

predict_coverage *Prediction of Primer Coveragee.*

Description

Predicts primer coverage using a logistic regression model. Converts coverage probabilities to expected false positive rate for a given probability.

Usage

```
predict_coverage(
  primer.df,
  template.df,
  settings,
  mode = c("on_target", "off_target"),
  updateProgress = NULL
)
```

Arguments

primer.df	A Primers data frame.
template.df	A Templates data frame.
settings	A DesignSettings object.
mode	Whether on-target or off-target events shall be considered.

Value

The predictions for primer coverage

`prefilter.primer.candidates` *Identication of Short Primers.*

Description

Identify initial primers that are too short.

Usage

```
prefilter.primer.candidates(primer.candidates, min.len)
```

Arguments

`primer.candidates`	Primer alignment.
`min.len`	Minimal primer length.

Value

The index of proposed primers that are shorter than `min.len`.

`prepare.constraint.plot` *Preparation of Comparison Plot for Evaluation.*

Description

Preparation of Comparison Plot for Evaluation.

Usage

```
prepare.constraint.plot(primer.data, constraint.settings, plot.p.vals = FALSE)
```

Arguments

`primer.data`	List with objects of class `Primers`. Each list entry corresponds to a single primer set.
`constraint.settings`	List with settings for each constraint. If `NULL` (the default), use the available evaluation results in `primer.data`.
`plot.p.vals`	Whether p-values from Fisher's exact test should be annotated for every primer set.

Value

Plot indicating the ratio of primers fulfilling the constraints specified in `constraint.settings` for each primer set in `primer.data`.

---

prepare.dimer.seqs *Preparation of Input for Dimerization.*

Description

Preparation of Input for Dimerization.

Usage

```
prepare.dimer.seqs(s1, s2)
```

Arguments

`s1`	Nucleotide character vectors (5' to 3')
`s2`	Nucleotide character vectors (5' to 3')

Value

A list with two fields containing character vectors.

---

prepare_mm_plot *Data Preparation for Mismatch Plot.*

Description

Data Preparation for Mismatch Plot.

Usage

```
prepare_mm_plot(primer.df, template.df, mode = c("on_target", "off_target"))
```

Arguments

`primer.df`	A `Primers` object.
`template.df`	A `Templates` object.
`mode`	Whether to compute for on-target or off-target events.

Value

A data frame with binding information for every primer.

prepare_template_cvg_mm_data

Preparation of Data for Plotting Mismatch Template Coverage.

Description

Creates a data frame for plotting a bar plot for the covered templates per allowed mismatches.

Usage

```
prepare_template_cvg_mm_data(primer.df, template.df, allowed.mismatches = NULL)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.
allowed.mismatches	An optional numeric specifying the number of mismatches to be considered at most for plotting. If not provided, the maximal number of mismatches found for the input primer set is used.

Value

A data frame for creating a plot.

primer.binding.regions.data

Primer Binding Region Data

Description

Collects all data concerning primer binding regions.

Usage

```
primer.binding.regions.data(
  primer.df,
  template.df,
  direction = c("both", "fw", "rev"),
  group = NULL,
  relation = c("fw", "rev")
)
```

Arguments

primer.df	Primer data frame.
template.df	Template data frame.
direction	Primer direction
group	The groups for which binding data shall be retrieved.
relation	Binding region data relative to forward/reverse binding region?

Value

Data frame with primer binding data.

---

primer.coverage.for.groups

Determination of Primer Coverage for Groups.

Description

Modifies a primer data frame to retain only coverage events relating to the selected groups of templates.

Usage

```
primer.coverage.for.groups(primer.df, template.df, groups)
```

Arguments

primer.df	Primer data frame.
template.df	Template data frame.
groups	Template groups for which coverage should be determined.

Value

primer.df with coverage considered only for groups.

primer.set.parameter.stats *Primer Set Statistics*

Description

Creates an overview of all primer set constraint values.

Usage

```
primer.set.parameter.stats(primer.df, mode.directionality, lex.seq)
```

Arguments

primer.df	Primer data frame.
mode.directionality	Direction of primers.
lex.seq	Template data frame.

Value

A data frame with statistics.

PrimerDesign *Primer Design Functionalities.*

Description

`design_primers` Designs a primer set maximizing the number of covered templates using the smallest possible number of primers. The algorithm tries to ensure that the designed set of primers achieves a coverage ratio not lower than `required.cvg`. To this end, the constraints for designing primers may be relaxed.

`get_initial_primers` Creates a set of primer candidates based on the input template sequences. This set of primers can be used to create custom primer design algorithms.

Usage

```
classify_design_problem(
  template.df,
  mode.directionality = c("both", "fw", "rev"),
  primer.length = 18,
  primer.estimate = FALSE,
  required.cvg = 1
)
```

```
get_initial_primers(
  sample,
  template.df,
  primer.lengths,
  mode.directionality = c("fw", "rev"),
  allowed.region.definition = c("within", "any"),
  init.algo = c("naive", "tree"),
  max.degen = 16,
  conservation = 1,
  updateProgress = NULL
)

design_primers(
  template.df,
  mode.directionality = c("both", "fw", "rev"),
  settings,
  init.algo = c("naive", "tree"),
  opti.algo = c("Greedy", "ILP"),
  required.cvg = 1,
  timeout = Inf,
  max.degen = 16,
  conservation = 1,
  sample.name = NULL,
  cur.results.loc = NULL,
  primer.df = NULL,
  updateProgress = NULL
)
```

Arguments

`template.df`	A `Templates` object containing the template sequences with annotated primer target binding regions.
`mode.directionality`	The template strand for which primers shall be designed. Primers can be designed either for forward strands ("fw"), for reverse strands ("rev"), or for both strands ("both"). The default setting is "both".
`primer.length`	A scalar numeric providing the target length of the designed primers. The default length of generated primers is set to 18.
`primer.estimate`	Whether the number of required primers shall be estimated. By default (`FALSE`), the number of required primers is not estimated.
`required.cvg`	The desired ratio of of covered template sequences. If the target coverage ratio cannot be reached, the constraint settings are relaxed according to the the constraint limits in order to reach the target coverage. The default `required.cvg` is set to 1, indicating that 100% of the templates are to be covered.
`sample`	Character vector providing an identifier for the templates.
`primer.lengths`	Numeric vector of length 2 providing the minimal and maximal allowed lengths for generated primers.

allowed.region.definition
: A character vector providing the definition of region where primers are to be constructed. If allowed.region.definition is "within", constructed primers lie within the allowed binding region. If allowed.region.definition is "any", primers overlap with the allowed binding region. The default is "within".

init.algo
: The algorithm to be used for initializing primers. If init.algo is "naive", then primers are constructed from substrings of the input template sequences. If init.algo is "tree", phylogenetic trees are used to form degenerate primers whose degeneracy is bounded by max.degen. This option requires an installation of MAFFT (see notes). The default init.algo is "naive".

max.degen
: The maximal degeneracy of primer candidates. This setting is particularly relevant when init.algo is set to "tree". The default setting is 16, which means that at most 4 maximally degenerate positions are allowed per primer.

conservation
: Restrict the percentile of considered regions according to their conservation. Only applicable for the tree-based primer initialization. At the default of 1, all available binding regions are considered.

updateProgress
: Shiny progress callback function. The default is NULL such that no progress is logged.

settings
: A DesignSettings object specifying the constraint settings for designing primers.

opti.algo
: The algorithm to be used for solving the primer set covering problem. If opti.algo is "Greedy" a greedy algorithm is used to solve the set cover problem. If opti.algo is "ILP" an integer linear programming formulation is used. The default opti.algo is "Greedy".

timeout
: Timeout in seconds. Only applicable when opti.algo is "ILP". The default is Inf, which does not limit the runtime.

sample.name
: An identifier for the primer design task. The default setting is NULL, which means that the run identifier provided in template.df is used.

cur.results.loc
: Directory for storing the results of the primer design procedure. The default setting is NULL such that no output is stored.

primer.df
: An optional Primers object. If an evaluated primer.df is provided, the primer design procedure only optimizes primer.df and does not perform the initialization and filtering steps. The default is NULL such that primers are initialized and filtered from scratch.

Details

classify_design_problem determines the difficulty of a primer design task by estimating the distribution of coverage ratios per primer by performing exact string matching with primers of length primer.length, which are constructed by extracting template subsequences. Next, a beta distribution is fitted to the estimated coverage distribution, which is then compare to reference distributions representing primer design problems of different difficulties via the total variance distance. The difficulty of the input primer design problem is found by selecting the class of the reference distributions that has the smallest distance to the estimated coverage distribution. An estimate of the required number of primers to reach a given required.cvg can be computed by setting

primer.estimate to TRUE. Since this estimate is based solely on perfect matching primers, the number of primers that would actually be required is typically less.

The primer design algorithm used by design_primers consists of three steps: primer initialization, filtering, and optimization. The method for initializing a set of candidate primers is determined via init.algo. If init.algo is set to *naive*, primers are created by extracting substrings from all input template sequences. If init.algo is set to *tree*, degenerate primers are created by merging similar subsequences by forming their consensus sequence up to a degeneracy of at most max.degen. The tree-based initialization is recommended for related sequences.

The candidate primer set is filtered according to the constraints specified in the settings object. In some cases, it is necessary to relax the constraints in order to reach the desired required.cvg. In these cases, primers that fail the input constraints may be selected. If you would like to skip the initialization and filtering stages, you can provide an evaluated Primers object via primer.df.

Optimizing a primer set entails finding the smallest subset of primers maximizing the coverage, which is done by solving the set cover problem. If melting temperature differences are a constraint, the optimization procedure automatically samples ranges of melting temperatures to find optimal sets for all possible temperatures. You can select the used optimization algorithm via optia.algo, where you can set "Greedy" for a greedy algorithm or "ILP for an integer linear program formulation (ILP). While the worst-case runtime of the greedy algorithm is shorter than the worst-case runtime of the ILP, the greedy solution may yield larger primer sets than the ILP solution.

Value

classify_design_problem returns a list with the following fields:

Classification The estimated difficulty of the primer design task.

Class-Distances The total variance distance of the fitted beta distribution to the reference distribution.

Confidence The confidence in the estimate of the design tasks' difficulty as based on the class distances.

Uncertain Whether the classification is highly uncertain, that is low-confidence.

Nbr_primers_fw **and** Nbr_primers_rev The respective number of required forward and reverse primers if primer.estimate was set to TRUE.

get_initial_primers returns a data frame with candidate primers for optimization.

design_primers returns a list with the following fields:

opti**:** A Primers object providing the designed primer set.

used_constraints**:** A list with DesignSettings objects for each primer direction providing the (possibly relaxed) constraints used for designing the optimal primers.

all_results**:** A list containing objects of class Primers. Each list entry corresponds to an optimal primer set for a given melting temperature.

all_used_constraints**:** A list containing DesignSettings object for each optimized set in all_results.

filtered**:** A list with data providing information on the results of the filtering procedure.

Note

Some constraints can only be computed if additional software is installed, please see the documentation of DesignSettings for more information. The usage of `init.algo = "tree"` requires an installation of the multiple alignment program MAFFT (http://mafft.cbrc.jp/alignment/software/).

Examples

```
data(Ippolito)
# Naive primer initialization
init.primers <- get_initial_primers("InitialPrimers", template.df,
                        c(18,18), "fw", init.algo = "naive")
# Tree-based primer initialization (requires MAFFT)
## Not run:
init.primers <- get_initial_primers("InitialPrimers", template.df,
                        c(18,18), "fw", init.algo = "tree")

## End(Not run)

# Define PCR settings and primer criteria
data(Ippolito)
# design only with minimal set of constraints
constraints(settings)$primer_length <- c("min" = 18, "max" = 18)
constraints(settings) <- constraints(settings)[c("primer_length", "primer_coverage")]
# Design only forward primers using a greedy algorithm
optimal.primers.greedy <- design_primers(template.df[1:2,], "both", settings, init.algo = "naive")
# Usage of the tree-based initialization strategy (requires MAFFT)
## Not run:
out.dir <- tempdir()
optimal.primers.tree <- design_primers(template.df[1:2,], "both", settings,
                        init.algo = "tree", opti.algo = "ILP",
                        max.degen = 16,
                        cur.results.loc = out.dir)

## End(Not run)
```

`PrimerEval` *Primer Evaluation.*

Description

`check_constraints` Determines whether a set of primers fulfills the constraints on the properties of the primers.

`check_restriction_sites` Checks a set of primers for the presence of restriction sites. To reduce the number of possible restriction sites, only unambiguous restriction sites are taken into account and only common (typically used) restriction sites are checked if a common restriction site can be found in a sequence.

`filter_primers` Filters a primer set according to the specified constraints such that all primers that do not fulfill the constraints are removed from the primer set.

`primer_significance` Uses Fisher's exact test to determine the significance of a primer set according to its ratio of fulfilled constraints.

`subset_primer_set` Determines subsets of the input primer set that are optimal with regard to the number of covered template sequences.

Usage

```
check_restriction_sites(
  primer.df,
  template.df,
  adapter.action = c("warn", "rm"),
  selected = NULL,
  only.confident.calls = TRUE,
  updateProgress = NULL
)

check_constraints(
  primer.df,
  template.df,
  settings,
  active.constraints = names(constraints(settings)),
  to.compute.constraints = active.constraints,
  for.shiny = FALSE,
  updateProgress = NULL
)

filter_primers(
  primer.df,
  template.df,
  settings,
  active.constraints = names(constraints(settings))
)

subset_primer_set(
  primer.df,
  template.df,
  k = 1,
  groups = NULL,
  identifier = NULL,
  cur.results.loc = NULL
)

primer_significance(primer.df, set.name = NULL, active.constraints = NULL)
```

Arguments

`primer.df`	A `Primers` object containing the primers whose properties are to be checked.
`template.df`	A `Templates` object containing the template sequences corresponding to `primer.df`.

`adapter.action` The action to be performed when adapter sequences are found. Either "warn" to issue a warning about adapter sequences or "rm" to remove identified adapter sequences. Currently, only the default setting ("warn") is supported.

`selected` Names of restriction sites that are to be checked. By default `selected` is `NULL` in which case all REBASE restriction sites are taken into account.

`only.confident.calls`
Whether only confident calls of restriction sites are returned. All restriction site call is considered *confident* if the restriction site is located in a region that does not match the template sequences. Note that this classification requires that the provided primers are somehow complementary to the provided templates. In contrast, non-confident restriction site calls are based solely on the primer sequences and do not take the templates into account, resulting in more false positive calls of restriction sites.

`updateProgress` Progress callback function for shiny. The defaut is `NULL` meaning that no progress is monitored via the Shiny interface.

`settings` A `DesignSettings` object containing the constraints that are to be considered.

`active.constraints`
A subset of the constraint identifiers provided by `settings` that are to be checked for fulfillment. By default `active.constraints` is `NULL` such that all constraints found in `settings` are evaluated. Otherwise, only the constraints specified via `active.constraints` that are available in `settings` are considered.

`to.compute.constraints`
Constraints that are to be computed. By default, `to.compute.constraints` is set to `NULL` such that all `active.constraints` are computed. If `to.compute.constraints` is a subset of `active.constraints`, all constraints specified via `active.constraints` are evaluated for fulfillment, but only the constraints in `to.compute.constraints` are newly calculated.

`for.shiny` Whether the output of the function shall be formatted as HTML. The default setting is `FALSE`.

`k` The spacing between generated primer subset sizes. By default, `k` is set to 1 such that all primer subsets are constructed.

`groups` The identifiers of template groups according to which coverage should be determined. By default, `groups` is set to `NULL` such that all all covered templates are considered.

`identifier` An identifier for storing the primer set. By default, `identifier` is set to `NULL`.

`cur.results.loc`
Directory for storing the results. By default, `cur.results.loc` is set to `NULL`, which means that no results are stored.

`set.name` An identifier for the input primers. If `NULL`, the run identifier is used.

Details

When the optional argument `active.constraints` is supplied to `check_constraints`, only a subset of the constraints provided in `settings` is evaluated. Only constraints that are defined in `settings` can be computed. For a detailed description of all possible constraints and their options, please consider the `ConstraintSettings` documentation.

subset_primer_set determines optimal subsets of the input primer set by solving an integer-linear program. Since the quality of the primers (in terms of properties) is not taken into account when creating the subsets, this method should only be used for primer sets that are already of high quality.

primer_significance computes the significance by comparing the total count of fulfilled and failed constraints with the corresponding counts of primer sets from the literature. Significant p-values indicate primer sets whose rate of constraint fulfillment is higher compared to the reference sets.

Value

check_restriction_sites returns a data frame with possible restriction sites found in the primers.

check_constraints returns a Primers object that is augmented with columns providing the results for the evaluated constraints. The constraints_passed column indicates whether all active.constraints were fulfilled. The EVAL_* columns indicate the fulfillment of primer-specific constraints. The T_EVAL_* columns indicate the fulfillment of template-specific (e.g. coverage-based) constraints. For the coverage computations, columns prefixed by Basic_, indicate the results from string matching, while all other results (e.g. primer_coverage) indicate the expected coverage after applying the coverage constraints specified in settings. Columns prefixed by Off_ indicate off-target binding results.

filter_primers returns a Primers object containing only those primers fulfilling all specified constraints.

subset_primer_set returns a list with optimal primer subsets, each of class Primers.

primer_significance returns a numeric providing the p-value of the primer set according to Fisher's exact test. The returned value has the following attributes:

test The results of the significance test

tab The confusion matrix for Fisher's exact test

constraints The names of the considered constraints

Note

Please note that some constraint computations may require the installation of additional programs; for more information please view the documentation of DesignSettings.

References

Roberts, R.J., Vincze, T., Posfai, J., Macelis, D. (2010) REBASE–a database for DNA restriction and modification: enzymes, genes and genomes. Nucl. Acids Res. 38: D234-D236. http://rebase.neb.com

Examples

```
data(Ippolito)
# Check the first primer for restriction sites with respect to the first 10 templates
site.df <- check_restriction_sites(primer.df[1,], template.df[1:10])

data(Ippolito)
settings.xml <- system.file("extdata", "settings",
                 "C_Taq_PCR_high_stringency.xml", package = "openPrimeR")
```

```
settings <- read_settings(settings.xml)
# Check GC clamp and number of runs for all primers:
constraint.df <- check_constraints(primer.df, template.df,
                     settings, active.constraints = c("gc_clamp", "no_runs"))
# Summarize the evaluation results
summary(constraint.df)

data(Ippolito)
filename <- system.file("extdata", "settings",
             "C_Taq_PCR_high_stringency.xml", package = "openPrimeR")
settings <- read_settings(filename)
# Only retain the primers fulfilling the GC clamp constraint:
filtered.df <- filter_primers(primer.df, template.df, settings,
                 active.constraints = c("gc_ratio"))

# Determine optimal primer subsets
data(Ippolito)
primer.subsets <- subset_primer_set(primer.df, template.df, k = 3)

# Determine the significance of a primer set
data(Ippolito)
p.data <- primer_significance(primer.df, "Ippolito")
attr(p.data,"tab") # the confusion matrix
attr(p.data, "test") # results from Fisher's test
attr(p.data, "constraints") # considered constraints for the test
```

`rbind.primer.data` *Rbind for Primer Data Frames.*

Description

Merges all primer data frames in `primer.data` into one data frame.

Usage

```
## S3 method for class 'primer.data'
rbind(primer.data)
```

Arguments

`primer.data`	List with primer data frames.

Value

A data frame containing all data in `primer.data`.

rbind.Primers *rbind for Primers class.*

Description

Ensures that the rbind result has the appropriate class.

Usage

```
## S3 method for class 'Primers'
rbind(...)
```

Arguments

...	Parameters for rbind function.

Value

Row-binded Primers data frame.

Examples

```
data(Ippolito)
primer.df <- rbind(primer.df, primer.df)
```

rbind.Templates *rbind for Template class.*

Description

Ensures that the rbind result has the appropriate class.

Usage

```
## S3 method for class 'Templates'
rbind(...)
```

Arguments

...	Parameters for rbind function.

Value

Row-binded Templates data frame.

Examples

```
data(Ippolito)
template.df <- rbind(template.df, template.df)
```

read.leaders *Read Individual Binding Regions*

Description

Reads individual binding regions into a data frame.

Usage

```
read.leaders(
  fasta.file,
  direction = c("fw", "rev"),
  rm.keywords = NULL,
  gap.character
)
```

Arguments

fasta.file	Path to a FASTA file with binding regions.
direction	String identifying whether the FASTA file contains information pertaining to the binding region of forward or reverse primers.
rm.keywords	A vector of keywords that are used to remove templates whose headers contain any of the keywords.
gap.character	The character for indicating gaps in sequences.

Value

A data frame with individual binding regions.

read.secondary.structure.raw *Read a Secondary Structure*

Description

Reads the secondary structure output of ViennaRNA.

Usage

```
read.secondary.structure.raw(fw.out)
```

Arguments

fw.out	Path to a ViennaRNA secondary structure output file.

Value

Data frame with information on secondary structures.

`read.sequences` *Read Sequences.*

Description

Reads an input FASTA file.

Usage

```
read.sequences(fasta.file, gap.character)
```

Arguments

`fasta.file`	The path to a FASTA file.
`The`	character indicating gaps in sequences.

Value

A data frame with sequences.

`read_primers.internal` *Internal Function for Reading Primers*

Description

Reads the given primer sequences into a data frame.

Usage

```
read_primers.internal(
  primer.seqs,
  headers,
  fw.id,
  rev.id,
  merge.ambig = c("none", "merge", "unmerge"),
  max.degen,
  sample.name
)
```

Arguments

primer.seqs	Primer sequences.
headers	Headers of the primer FASTA file.
fw.id	Identifier of forward primers in the headers.
rev.id	Identifier of reverse primers in the headers.
merge.ambig	Should ambiguous primers be merged?
max.degen	Maximum allowed degeneracy

Value

A data frame with primer sequences.

---

read_primers_csv *Read Primer CSV File.*

Description

Reads a primer data frame stored in a CSV file.

Usage

```
read_primers_csv(file)
```

Arguments

file	The path to a csv file containing the primer data.

Value

A Primers object, an instance of a data frame.

---

read_primers_multiple *Input of Multiple Primer Sets.*

Description

Reads multiple CSV files representing stored objects of class Primers.

Usage

```
read_primers_multiple(
  filenames,
  fw.id,
  rev.id,
  merge.ambig,
  max.degen,
  template.df,
  adapter.action,
  sample.name,
  updateProgress
)
```

Arguments

filenames	The paths to multiple primer CSV/FASTA files.

Value

A list containing objects of class Primers.

---

read_templates_csv *Read Template CSV File*

Description

Reads an input template CSV file.

Usage

```
read_templates_csv(fname)
```

Arguments

fname	The filename of the input template CSV file.

Value

A template data frame.

`read_templates_fasta` *Input of Template Sequences.*

Description

Read template sequences from a FASTA file.

Usage

```
read_templates_fasta(
  fasta.file,
  hdr.structure = NULL,
  delim = NULL,
  id.column = NULL,
  rm.keywords = NULL,
  remove.duplicates = FALSE,
  fw.region = c(1, 30),
  rev.region = c(1, 30),
  gap.character = "-",
  run = NULL
)
```

Arguments

`fasta.file`	Path to a FASTA file containing the template sequences.
`hdr.structure`	Names describing the information contained in the FASTA headers. In case that the headers of `fasta.file` contain template group information, please include the keyword "GROUP" in `hdr.structure`.
`delim`	Delimiter for the information in the FASTA headers.
`id.column`	Field in the header to be used as the identifier.
`rm.keywords`	A vector of keywords that are used to remove templates whose headers contain any of the keywords.
`remove.duplicates`	Whether duplicate sequence shall be removed.
`fw.region`	The positional interval from the template 5' end specifying the binding sites for forward primers.
`rev.region`	The positional interval from the template 3' end specifying the binding sites for reverse primers.
`gap.character`	The character in the input file representing gaps. Gaps are automatically removed upon input.
`run`	An identifier for the template sequences.

Value

An object of class `Templates`.

Examples

```
fasta.file <- system.file("extdata", "IMGT_data", "templates",
              "Homo_sapiens_IGH_functional_exon.fasta", package = "openPrimeR")
hdr.structure <- c("ACCESSION", "GROUP", "SPECIES", "FUNCTION")
template.df <- read_templates(fasta.file, hdr.structure, "|", "GROUP")
```

`read_templates_multiple` *Input of Multiple Templates.*

Description

Reads multiple template CSV/FASTA files.

Usage

```
read_templates_multiple(
  filenames,
  hdr.structure = NULL,
  delim = NULL,
  id.column = NULL,
  rm.keywords = NULL,
  remove.duplicates = FALSE,
  fw.region = c(1, 30),
  rev.region = c(1, 30),
  gap.character = "-",
  run = NULL
)
```

Arguments

`filenames`	Names of FASTA/CSV files containing template data.
`hdr.structure`	A character vector describing the information contained in the FASTA headers. In case that the headers of `fasta.file` contain template group information, please include the keyword "GROUP" in `hdr.structure`.
`delim`	Delimiter for the information in the FASTA headers.
`id.column`	Field in the header to be used as the identifier.
`rm.keywords`	A vector of keywords that are used to remove templates whose headers contain any of the keywords.
`remove.duplicates`	Whether duplicate sequence shall be removed.
`fw.region`	The positional interval from the template 5' end specifying the binding sites for forward primers.
`rev.region`	The positional interval from the template 3' end specifying the binding sites for reverse primers.

gap.character	The character in the input file representing gaps. Gaps are automatically removed upon input.
run	An identifier for the template sequences.

Value

A list containing objects of class Templates.

`read_templates_single` *Input of a Single Template File.*

Description

Read template sequences from a FASTA or CSV file.

Usage

```
read_templates_single(
  template.file,
  hdr.structure = NULL,
  delim = NULL,
  id.column = NULL,
  rm.keywords = NULL,
  remove.duplicates = FALSE,
  fw.region = c(1, 30),
  rev.region = c(1, 30),
  gap.character = "-",
  run = NULL
)
```

Arguments

template.file	Path to a FASTA or CSV file containing the template sequences.
hdr.structure	A character vector describing the information contained in the FASTA headers. In case that the headers of fasta.file contain template group information, please include the keyword "GROUP" in hdr.structure.
delim	Delimiter for the information in the FASTA headers.
id.column	Field in the header to be used as the identifier.
rm.keywords	A vector of keywords that are used to remove templates whose headers contain any of the keywords.
remove.duplicates	Whether duplicate sequence shall be removed.
fw.region	The positional interval from the template 5' end specifying the binding sites for forward primers.

rev.region	The positional interval from the template 3' end specifying the binding sites for reverse primers.
gap.character	The character in the input file representing gaps. Gaps are automatically removed upon input.
run	An identifier for the template sequences.

Details

When supplying a FASTA file with template sequences, the input arguments hdr.structure, delim, id.column, rm.keywords, remove.duplicates, fw.region, rev.region, gap.character, and run are utilized. Most importantly, hdr.structure and delim should match the FASTA header structure. When supplying a CSV file with template sequences, the data are loaded without any modification and the CSV file should represent an object of class Templates, which can be stored using the write_templates function.

Value

An object of class Templates.

---

relax.constraints *Relaxation of Constraints*

Description

Relax constraints trying to reach the target coverage ratio.

Usage

```
relax.constraints(
  settings,
  filtered.df,
  excluded.df,
  stat.df,
  template.df,
  mode.directionality = c("fw", "rev"),
  required.cvg,
  target.temps = NULL,
  results.loc = NULL
)
```

Arguments

settings	A DesignSettings object.
filtered.df	Data set of primers that fulfilled all constraints of the filtering procedure.
excluded.df	Data frame with excluded primers from the first filtering round.
stat.df	Data frame with statistics from filtering.

`template.df` Template data frame.

`mode.directionality` Primer direction.

`required.cvg` Required ratio of covered templates.

`target.temps` Target melting temperature values.

`results.loc` The location where intermediary results should be stored.

Value

A list containing information about the relaxation as well as the filtered primers.

`relax.opti.constraints`

Relaxation of Optimization Constraints

Description

Relax optimization constraints.

Usage

```
relax.opti.constraints(
  cur.opti.constraints,
  initial.opti.limits,
  initial.opti.constraints
)
```

Arguments

`cur.opti.constraints` List with optimization constraint settings.

`initial.opti.limits` Initial optimization limits.

`initial.opti.constraints` Initial optimization constraints.

Value

Relaxed optimization constraints.

`remove.redundant.cols` *Removal of Redundant Primers.*

Description

Removes redundant primers from an optimal solution.

Usage

```
remove.redundant.cols(S, cvg.matrix)
```

Arguments

`S`	Indices of primers that are selected in an optimal solution.
`cvg.matrix`	Binary matrix of coverage events.

Details

An optimal solution can contain primers with redundant coverage when using presolve or greedy optimization.

Value

Updated indices of selected primers `S` such that indices representing primers with redundant coverage are removed.

`remove.seqs.by.keyword`
Removal of Partial Sequences.

Description

Removes partial template sequences.

Usage

```
remove.seqs.by.keyword(template.df, keyword = "partial")
```

Arguments

`template.df`	Template data frame.
`Header`	keywords indiating templates that should be excluded.

Value

Template data frame with partial sequences removed.

rename.constraint.options *Renaming of Constraint Options.*

Description

Renames the input list with constraint options.

Usage

```
rename.constraint.options(constraint.options)
```

Arguments

constraint.options	A list with constraint options.

Value

A list with renamed constraint options.

render_report *Renders an rmarkdown file using Pandoc.*

Description

Creates a PDF report using rmarkdown and Pandoc by passing the specified params to the markdown file given by report_template and storing the PDF in out.file.

Usage

```
render_report(params, report_template, out.file)
```

Arguments

params	A list with parameters for the R markdown parser.
report_template	A character vector giving the basename of the Rmarkdown template to use for report creation.
out.file	The filename of the report PDF to be created.

Value

Creates a PDF in out.file if successful.

reorder.primer.table *Reorder Primers*

Description

Reorders a primer set according to the IDs of primers.

Usage

```
## S3 method for class 'primer.table'
reorder(filtered.primers, primer.ID.order)
```

Arguments

filtered.primers	Primer data frame.
primer.ID.order	new ordering of IDs in the data frame.

Value

Reordered primer data frame.

restriction_ali *Identification of Badly Fitting Regions.*

Description

Identify regions in the templates where the primers are not very complementary. These regions indicate possible restriction enzyme adapters.

Usage

```
restriction_ali(primer.seqs, template.seqs, search.hits)
```

Arguments

primer.seqs	Primer sequences.
template.seqs	Template sequences.
search.hits	Template substrings that agree well with the input primers.

Value

A list with putative restriction sites for every primer.

restriction_hits *Identification of Restriction Sites.*

Description

Identifies restriction sites in a list with putative restriction sites provided by `bad.regions` using a data frame of restriction sites given by `DB`.

Usage

```
restriction_hits(bad.regions, DB)
```

Arguments

`DB`	A data frame with restriction enzyme sites.
`bad.region`	IRanges with possible adapter sites.

Value

A boolean data frame indicating the presence of adapters for all considered restriction sites.

restriction_match *Identification of Sequence Matches.*

Description

Determines the most similar template sequence for every input primer sequence. Used to identify regions for alignment for the identification of restriction sites.

Usage

```
restriction_match(primer.seqs, template.seqs)
```

Arguments

`primer.seqs`	Primer sequences.
`template.seqs`	Template sequences.

Value

A vector with the template regions matching the `primer.seqs` best.

retrieve.leader.region *Retrieval of Binding Regions*

Description

Retrives information about individual binding regions.

Usage

```
retrieve.leader.region(
  template.df,
  direction = c("fw", "rev"),
  start,
  end,
  gap.char,
  init.from.leader
)
```

Arguments

`template.df`	Template data frame.
`direction`	Identify forward and reverse.
`start`	Start positions.
`end`	End positions.
`gap.char`	The character for gaps in alignments.
`init.from.leader`	Whether the binding regions are initialized from leader sequences.

Value

Data frame with information on allowed binding regions.

rev.comp.sequence *Reverse complement of a sequence*

Description

Computes the reverse complement of the input sequences.

Usage

```
## S3 method for class 'comp.sequence'
rev(seq)
```

Arguments

seq	the input strings

Value

The reverse complement of the input sequences.

---

rev.sequence *Reversion of a sequence*

Description

Reverses the input sequences.

Usage

```
## S3 method for class 'sequence'
rev(seq)
```

Arguments

seq	the input sequence.

Value

The input sequence in reverse order.

---

runTutorial *The openPrimeR Tutorial.*

Description

Starts a Shiny app containing the openPrimeR tutorial, which was built using the learnr package. The application starts locally and should open a new tab in your default browser. If no browser is opened, please consider the console output to identify the local port on which the server is running.

Usage

```
runTutorial(dev = FALSE)
```

Arguments

dev	A logical indicating whether to start the development version of the tutorial (default: FALSE).

Value

Opens the openPrimeR tutorial in a web browser.

Note

The Shiny app can be started only if you fulfill all of the suggested package dependencies for the Shiny framework, so please ensure that you've installed openPrimeR including all suggested dependencies.

Examples

```
## Not run:
# Open the tutorial
if (interactive()) {
runTutorial()
}

## End(Not run)
```

---

sanitize_path *Sanitiziation of Filename.*

Description

Ensures that a filename is valid for the file system.

Usage

```
sanitize_path(path, suffix = "", sub.char = "_")
```

Arguments

path	The path to the file to be sanitized, without file extension.
suffix	The suffix (e.g. ".png") of a file.
sub_char	The character used to replacing disallowed chars.

Value

The sanitized filename

score.conservation *Conservation Scores*

Description

Scores the conservation of alignment regions.

Usage

```
score.conservation(primer.range, ali.entropy)
```

Arguments

primer.range	A data frame with starts/ends of primers.
ali.entropy	Entropies corresponding to the alignment

Value

Entropies indicating conservation (similarity) of regions.

Scoring *Scoring Functions.*

Description

score_degen Determines the degeneration score of a sequence.

score_conservation Determines the sequence conservation scores of a set of templates using Shannon entropy.

score_primers Computes scores for a set of primers based on the deviations of the primers from the constraints.

Usage

```
score_conservation(template.df, gap.char = "-", win.len = 30, by.group = TRUE)

score_degen(seq, gap.char = "-")

score_primers(
  primer.df,
  settings,
  active.constraints = names(constraints(settings)),
  alpha = 0.5
)
```

Arguments

template.df	A Templates object providing the set of templates.
gap.char	The gap character in the sequences. The default is "-".
win.len	The size of a window for evaluating conservation. The default window size is set to 30.
by.group	Whether the determination of binding regions should be stratified according to the groups defined in template.df. The default is TRUE.
seq	A list of vectors containing individual characters of a nucleotide sequence.
primer.df	A Primers object containing the primers.
settings	A DesignSettings object containing the analysis settings.
active.constraints	A character vector of constraint identifiers that are considered for scoring the primers.
alpha	A numeric that is used to determine the trade-off between the impact of the maximal observed deviation and the total deviation. At its default alpha is set to 0.5 such that the maximal deviation and the total deviation have an equal weight when computing the penalties.

Details

score_degen computes the degeneration of an ambiguous sequence by considering the number of unambiguous sequences that are represented by the the ambiguous sequence. Let a sequence S of length n be represented by a collection of sets such that

$$S = s_1, s_2, \ldots, s_n$$

where s_i indicates the set of unambiguous bases found at position i of the primer. Then the degeneracy D of a primer can be defined as

$$D = \prod_i |s_i|$$

where $|s_i|$ provides the number of disambiguated bases at position i.

score_primers determines the penalty of a primer in the following way. Let d be a vector indicating the absolute deviations from individual constraints and let p be the scalar penalty that is assigned to a primer. We define

$$p = \alpha \cdot \max_i d_i + \sum_i (1 - \alpha) \cdot d_i$$

such that for large values of alpha the maximal deviation dominates giving rise to a local penalty (reflecting the largest absolute deviation) and for small alpha the total deviation dominates giving rise to a global penalty (reflecting the sum of constraint deviations). When alpha is 1 only the most extreme absolute deviation is considered and when alpha is 0 the sum of all absolute deviations is computed.

Value

A list containing `Entropies` and `Alignments`. `Entropies` is a data frame with conservation scores. Each column indicates a position in the alignment of template sequences and each row gives the entropies of the sequences belonging to a specific group of template sequences. `Alignments` is a list of `DNABin` objects, where each object gives the alignment corresponding to one group of template sequences.

`score_degen` finds the number of unambiguous sequences that are represented by `seq`.

`score_primers` returns a data frame containing scores for individual primers.

Note

`score_conservation` requires the MAFFT software for multiple alignments (http://mafft.cbrc.jp/alignment/software/).

Examples

```
## Not run:
data(Ippolito)
entropy.data <- score_conservation(template.df, gap.char = "-", win.len = 18, by.group = TRUE)

## End(Not run)
# Compute degeneration for sequences with differing number of ambiguous bases
seq <- strsplit(c("ctggaattacggtacc", "taggaaccggrtaagc", "rtaaasrygtar"), split = "")
degen <- score_degen(seq)

# Score the primers
data(Ippolito)
primer.scores <- score_primers(primer.df, settings)
```

---

select.allowed.binding.events

Selection of Binding Events

Description

Selects primer binding events that are within the allowed binding regions.

Usage

```
select.allowed.binding.events(
  bound.fw,
  bound.to.allowed.region.fw,
  allowed.other.binding.ratio
)
```

Arguments

`bound.fw`	Indices of covered templates of a single primer.
`bound.to.allowed.region.fw`	Corresponding allowed binding regions.
`allowed.other.binding.ratio`	The ratio of other binding events. If this is different from 0, disallowed binding events will also be reported.

Value

The indices of the allowed binding events.

`select.best.ILP` *Selection of Best ILP*

Description

Selects the best solution from multiple solved ILP instances.

Usage

```
select.best.ILP(ILP.df)
```

Arguments

`ILP.df`	Data frame with ILP result properties.

Value

The index of the best solution.

`select.best.opti.result` *Selection of Best Greedy Result*

Description

Selects best greedy primer data set.

Usage

```
select.best.opti.result(opti.results, template.df)
```

Arguments

opti.results	List with primer data frames for different target melting temperatures.
template.df	Template data frame.

Value

The index of the best primer data set.

---

select.best.primer.idx *Greedy Choice*

Description

Selects the currently best primer for Greedy primer selection.

Usage

```
select.best.primer.idx(
  result,
  primers,
  deltaG.cutoff,
  target.temp,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc
)
```

Arguments

result	Data frame of current optimized primer data set that is to be augmented.
primers	Data frame of candidate primers for addition to result.
deltaG.cutoff	Free energy cutoff for cross-dimerization.
target.temp	Target annealing temperature in Celsius.
primer_conc	Primer concentration.
na_salt_conc	Sodium ion concentration.
mg_salt_conc	Magensium ion concentration.
k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris ion concentration.

Value

The index of a suitable primer according to Greedy selection.

select.best.primer.set *Selection of Best Primer Set.*

Description

Selects the best primer set according to coverage and melting temperature differences among primers in the set.

Usage

```
select.best.primer.set(stats)
```

Arguments

stats	Statistics of the primer sets to be evaluated.

Value

The index of the best primer set.

select.binding.events *Selection of Individual Binding Events*

Description

Selects only binding events of interest.

Usage

```
select.binding.events(fw.binding.filtered, p.idx)
```

Arguments

fw.binding.filtered	IRanges with binding events.
p.idx	Index of binding events to keep.

Value

An IRanges object containing only the selected binding events.

select.constraints *Selection of Constraints.*

Description

Selects constraints that can be computed according to installed third-party software. This function is only used for initializing the 'constraint_order' option.

Usage

```
select.constraints(active.constraints)
```

Arguments

active.constraints	A vector whose names give the constraints to be checked.

Value

A vector of useable constraint identifiers.

select.min.cross.idx *Selection of cross dimer index*

Description

Select the index with the smallest DeltaG value.

Usage

```
select.min.cross.idx(deltaG, primers)
```

Arguments

deltaG	Data frame with thermodynamic info.
primers	The corresponding primers.

Value

The indices with smallest DeltaG for every primer.

`select.primer.region.by.conservation` *Selection by Conservation*

Description

Selects primer regions for initialization of primers according to their conservation scores.

Usage

```
select.primer.region.by.conservation(
  primer.range,
  ali.entropy,
  conservation,
  bin,
  gap.char = "-"
)
```

Arguments

`primer.range`	Data frame with primer starts/stops.
`ali.entropy`	Entropy values for the alignment.
`conservation`	Desired ratio of primer conservation. Only regions with a conservation of at least `conservation` are considered for the initialization of primers.
`bin`	`DNABin` alignment of templates.
`gap.char`	The character for alignment gaps.

Details

The conservation scores are computed using the entropies computed from the alignment of the template sequence regions of interests.

Value

Updated primer regions according to the desired conservation.

`select.primers.by.cvg` *Greedy Optimization.*

Description

Greedy approach for solving the primer set coverage problem.

Usage

```
select.primers.by.cvg(
  primers,
  settings,
  template.df,
  mode.directionality = c("fw", "rev"),
  required.cvg = 1,
  allowed.mismatches,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  template_conc,
  allowed.other.binding.ratio,
  allowed.stop.codons,
  allowed.region.definition = c("within", "any"),
  disallowed.mismatch.pos,
  target.temps = NULL,
  fw.primers = NULL,
  updateProgress = NULL
)
```

Arguments

`primers`	Primer data frame to be optimized.
`settings`	A `DesignSettings` object.
`template.df`	Template data frame.
`mode.directionality`	Primer direction.
`required.cvg`	Target coverage ratio.
`allowed.mismatches`	The number of mismatches primers are allowed to have with the templates.
`primer_conc`	Primer concentration.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.

k_salt_conc	Potassium ion concentration.
tris_salt_conc	Tris ion concentration.
template_conc	Template data frame.
allowed.other.binding.ratio	Ratio of primers allowed to bind to non-target regions.
allowed.stop.codons	Consider mismatch binding events that induce stop codons.
allowed.region.definition	Definition of the target binding sites used for evaluating the coverage. If allowed.region.definition is within, primers have to lie within the allowed binding region. If allowed.region.definition is any, primers have to overlap with the allowed binding region. The default is that primers have to bind within the target binding region.
disallowed.mismatch.pos	The number of positions from the primer 3' end where mismatches should not be allowed. All primers binding templates with mismatches within disallowed.mismatch.pos from the 3' end are disregarded.
target.temps	Target melting temperatures for optimized sets in Celsius.
fw.primers	List with already optimized primer data frames corresponding to target.temps.
updateProgress	Shiny progress callback function.

Value

List with optimization data.

`select_best_binding` *Selection of Best (smallest number of mismatches) Binding Event per Template Coverage Event.*

Description

Selection of Best (smallest number of mismatches) Binding Event per Template Coverage Event.

Usage

```
select_best_binding(binding, fw.mm.info)
```

Arguments

binding	Binding information.
fw.mm.info	Info about mismatches.

Value

A list with entries 'fw' and 'rev' giving the best indices of primers.

selenium.installed *Determination if Selenium is installed.*

Description

Checks whether selenium module for python is installed on the system.

Usage

```
selenium.installed()
```

Value

TRUE is selenium for python is available, FALSE otherwise.

set.new.constraint.value *Update Constraint Settings.*

Description

Updates the constraint settings with new values. Sets to the maximal observed values within the limits or less if relax.speed is less than 1.

Usage

```
set.new.constraint.value(values, relax.speed, cur.limits, cur.setting)
```

Arguments

values	Observed constraint values considered for the update.
relax.speed	The speed at which the constraints should be relaxed. This value should be in the interval [0,1].
cur.limits	List with current relaxation limits.
cur.setting	List with current constraint settings.

Value

Relaxed constraint settings according to the given values and cur.limits.

set.new.limits *Relaxation of Constraint Limits.*

Description

Relaxes the constraint limits by moving according to the difference between the `initial.limits` and `initial.constraints`.

Usage

```
set.new.limits(
  cur.limits,
  initial.limits,
  initial.constraints,
  con.name = NULL
)
```

Arguments

`cur.limits`	List with current constraint settings.
`initial.limits`	List with initial coonstraint limits.
`initial.constraints`	List with initial constraint settings before relaxing.
`con.name`	The constraint for which the settings are to be changed.

Value

A list with relaxed constraint limits.

Settings *Settings Functionalities.*

Description

`DesignSettings` The `DesignSettings` class encapsulates all settings for designing and evaluating primer sets. Upon loading an XML file, the `DesignSettings` class checks whether the defined constraints can be applied by identifying whether the requirements for external programs are fulfilled. If the requirements are not fulfilled, the affected constraints are removed from the loaded `DesignSettings` object and a warning is issued. The loaded constraints are automatically ordered according to the option `openPrimeR.constraint_order` such that the runtime of the `design_primers` and `filter_primers` functions is optimized.

`constraints` Gets the active constraints of the provided `DesignSettings` object.

`constraints<-` Sets the active constraints of the provided `DesignSettings` object.

`cvg_constraints` Gets the coverage constraints of the provided `DesignSettings` object.

`cvg_constraints<-` Sets the coverage constraints of the provided `DesignSettings` object.

`conOptions` Gets the constraint settings of the provided `DesignSettings` object.

`conOptions<-` Sets the constraint settings of the provided `DesignSettings` object.

`constraintLimits` Gets the constraint limits that are defined in the provided `DesignSettings` object.

`constraintLimits<-` Sets the constraint limits of the provided `DesignSettings` object.

`PCR` Gets the PCR conditions that are defined in the provided `DesignSettings` object.

`PCR<-` Sets the PCR conditions that are defined in the provided `DesignSettings` object.

`ConstraintSettings` The `ConstraintSettings` class encapsulates the constraints on the physicochemical properties of primers.

`CoverageConstraints` The `CoverageConstraints` class encapsulates the conditions under which the coverage of primers is evaluated.

`PCR_Conditions` The `PCR_Conditions` class encapsulates the PCR conditions for the computation of primer properties.

`ConstraintOptions` The `ConstraintOptions` class encapsulates the options for constraint computations.

`parallel_setup` Registers the specified number of cores with the parallel backend.

Usage

```
constraints(x)

## S4 method for signature 'DesignSettings'
constraints(x)

## S4 method for signature 'AbstractConstraintSettings'
constraints(x)

cvg_constraints(x)

## S4 method for signature 'DesignSettings'
cvg_constraints(x)

PCR(x)

## S4 method for signature 'DesignSettings'
PCR(x)

conOptions(x)

## S4 method for signature 'DesignSettings'
conOptions(x)

constraintLimits(x)
```

```
## S4 method for signature 'DesignSettings'
constraintLimits(x)

constraints(x) <- value

## S4 replacement method for signature 'DesignSettings,list'
constraints(x) <- value

## S4 replacement method for signature 'AbstractConstraintSettings,list'
constraints(x) <- value

cvg_constraints(x) <- value

## S4 replacement method for signature 'DesignSettings'
cvg_constraints(x) <- value

constraintLimits(x) <- value

## S4 replacement method for signature 'DesignSettings'
constraintLimits(x) <- value

PCR(x) <- value

## S4 replacement method for signature 'DesignSettings'
PCR(x) <- value

conOptions(x) <- value

## S4 replacement method for signature 'DesignSettings'
conOptions(x) <- value

parallel_setup(cores = NULL)
```

Arguments

x	A DesignSettings object.
value	An object to be used in one of the setters. For constraints<- and constraintLimits<-, a list with constraint settings or boundaries. Each list entry should have a permissible name and consist of at most two values providing the minimal and/or maximal allowed values, which have to be denominated via min and max. For conOptions<-, a list with constraint options. The permissible fields of the list and their types are documented in the ConstraintOptions class. For cvg_constraints<-, a list with coverage constraints. Each list entry must have a permissible name and contain a numeric vector with at most two components describing the minimal and/or maximal required values that are to be indicated via min and max. The permissible contraint identifiers are documented in the CoverageConstraints class. For PCR<-, a named list providing PCR conditions The permissible fields of the

list and their types are documented in the PCR_Conditions class.

cores A numeric providing the number of cores to use. The default is NULL such that half the number of available cores are used.

Details

Note that for the DesignSettings class, the fields Input_Constraints, Input_Constraint_Boundaries, and Coverage_Constraints should contain entries with at most two components using the fields min and/or max. The Input_Constraint_Boundaries should always be at least as general as the specified Input_Constraints.

For an overview of permissible constraints, please consider the ConstraintSettings documentation.

Value

The ConstraintSettings constructor defines a new ConstraintSettings object.

The CoverageConstraints constructor initializes a new CoverageConstraints object.

The ConstraintOptions constructor returns a new ConstraintOptions object.

The PCR_Conditions constructor defines a new PCR_Conditions object.

The DesignSettings constructor defines a DesignSettings object.

constraints gets a list with the active constraint settings.

cvg_constraints returns the list of active coverage constraints.

PCR gets the list of PCR conditions defined in the provided DesignSettings object.

conOptions returns a list with constraint options.

constraintLimits gets the list of constraint limits.

constraints<- sets the list of constraints in a DesignSettings object.

cvg_constraints<- sets the list of coverage constraints in the provided DesignSettings object.

constraintLimits<- sets the list of constraint limits in the provided DesignSettings object.

PCR<- sets the constraint options in the provided DesignSettings object.

conOptions<- sets the specified list of constraint options in the provided DesignSettings object.

parallel_setup returns NULL.

Slots

Input_Constraints A ConstraintSettings object specifying the desired target value ranges for primer properties.

Input_Constraint_Boundaries A ConstraintSettings object specifying the limits for relaxing the constraints during the primer design procedure. This slot may contain the same fields as the Input_Constraints slot, but the specified desired ranges should be at least as general as those specified in the Input_Constraints slot.

Coverage_Constraints A CoverageConstraints object specifying the constraints for computing the primer coverage.

PCR_conditions A PCR_Conditions object specifying the PCR-related settings.

`constraint_settings` A `ConstraintSettings` object providing options for the computation of individual physicochemical properties.

`status` Named boolean vector indicating which of the possible constraints are active (`TRUE`) and which are not (`FALSE`).

`settings` For `ConstraintSettings`, a named list containing the settings for the active constraints. The list may contain the following fields:

primer_coverage: The required number of covered template sequences per primer.

primer_specificity: The required required specificity of primers in terms of a ratio in the interval [0,1].

primer_length: The required lengths of primer sequences.

gc_clamp: The desired number of GCs at primer 3' termini.

gc_ratio: The desired ratio of GCs in primers in terms of numbers in the interval [0,1].

no_runs: The accepted length homopolymer runs in a primer.

no_repeats: The accepted length of dinucleotide repeats in a primer.

self_dimerization: The lowest acceptable free energy [kcal/mol] for the interaction of a primer with itself. The identification of self dimers requires the software *OligoArrayAux* (see notes).

melting_temp_range: The desired melting temperature (Celsius) of primers. The accurate computation of melting temperatures requires the software *MELTING* (see notes).

melting_temp_diff: The maximal allowed difference between the melting temperatures (Celsius) of primers contained in the same set. The accurate computation of melting temperatures requires the software *MELTING* (see notes).

cross_dimerization: The lowest acceptable free energy [kcal/mol] for the interaction of a primer with another primer. The identification of cross dimers requires the software *OligoArrayAux* (see notes).

secondary_structure: The lowest acceptable free energy [kcal/mol] for the formation of primer secondary structures. Secondary structures are determined using the software *ViennaRNA* (see notes).

For `PCR_Conditions`, a named list with PCR conditions. The following fields are possible:

`use_taq_polymerase`**:** A logical identifying whether you are performing PCR with a Taq polymerase (`TRUE`) or not (`FALSE`).

`annealing_temp`**:** The annealing temperature in Celsius that is to be used for evaluating the constraints defined in the `ConstraintSettings` object. If the annealing temperature field is not provided, a suitable annealing temperature is automatically computed using a rule of thumb (i.e. subtracting 5 from the melting temperature).

`Na_concentration`**:** The molar concentration of monovalent sodium ions.

`Mg_concentration`**:** The molar concentration of divalent magnesium ions.

`K_concentration`**:** The molar concentration of monovalent potassium ions.

`Tris_concentration`**:** The molar concentration of the Tris(hydroxymethyl)-aminomethan buffer.

`primer_concentration`**:** The molar concentration of the PCR primers.

`template_concentration`**:** The molar concentration of the PCR templates.

For `CoverageConstraints`, a named list with constraint options. Each list entry should have an entry `min` and/or `max` in order to indicate the minimal and maximal allowed values, respectively. The following identifiers can be used as coverage constraints:

`primer_efficiency`**:** The desired efficiencies of primer-template amplification events in order to be considered as *covered*. `primer_efficiency` provides a value in the interval [0,1], which is based on **DECIPHER**'s thermodynamic model, which considers the impact of 3' terminal mismatches.

`annealing_DeltaG`**:** The desired free energies of annealing for putative coverage events between primers and templates. Typically, one would limit the maximally allowed free energy.

`stop_codon`**:** Whether coverage events introducing stop codons into the amplicons should be allowed or discarded. Here, a value of `1` indicates coverage events that induce stop codons. As such, setting both minimum and maximium to zero will disregard coverage events inducing stop codons, while setting the minimum to zero and the maximum to 1 will allow coverage events that induce stop codons.

`substitution`**:** Whether coverage events introducing substitutions into the amino acid sequence are considered or discarded. The same encoding as for `stop_codon` is used, that is, the value `1` indicates coverage events inducing substitutions. Hence, to prevent substitutions, the maximal value of `substitution` can be set to zero.

`terminal_mismatch_pos`**:** The position relative to the primer 3' terminal end for which mismatch binding events should be allowed, where the last base in a primer is indicated by position `1`. For example, setting the minimal value of `terminal_mismatch_pos` to `7` means that only coverage events that do not have a terminal mismatch within the last 6 bases of the primer are allowed.

`coverage_model`**:** Use a logistic regression model combining the free energy of annealing and 3' terminal mismatch positions to determine the expected rate of false positive coverage calls. Using `coverage_model`, you can specify the allowed ratio of falsely predicted coverage events. Typically, one would limit the maximal allowed rate of false positives. Note that setting a small false positive rate will reduce the sensitivity of the coverage calls (i.e. true positives will be missed).

For `ConstraintOptions`, a named list with constraint options. The following fields are permissible:

allowed_mismatches: The maximal number of allowed mismatches between a primer and a template sequence. If the number of mismatches of a primer with a template exceeds the specified value, the primer is not considered to cover the corresponding template when the coverage is being computed.

allowed_other_binding_ratio: Ratio of allowed binding events outside the target binding ratio. This value should be in the interval [0,1]. If the specified value is greater than zero, all coverage events outside the primer binding region are reported. If, however, the identified ratio of off-target events should exceed the allowed ratio, a warning is issued. If `allowed_other_binding_ratio` is set to `0`, only on-target primer binding events are reported. The setting of `allowed_other_binding_ratio` is ignored when designing primers, which always uses a value of 0.

allowed_region_definition: The definition of the target binding regions that is used for evaluating the coverage. In case that `allowed_region_definition` is `within`, primers have to lie within the allowed binding region. If `allowed_region_definition` is `any`, primers only have to overlap with the target binding region.

hexamer_coverage: If `hexamer_coverage` is set to "active", primers whose 3' hexamer (the last 6 bases) is fully complementary to the corresponding template region are automat-

ically considered to cover the template. If `hexamer_coverage` is set to `inactive`, hexamer complementarity does not guarantee template coverage.

primer_coverage

Computing the primer coverage involves identifying which templates are expected to be amplified (covered) by which primers. The `primer_coverage` constraint determines the minimal and maximal number of coverage events per primer that are required. The computation of primer coverage is governed by the coverage constraints postulated via `CoverageConstraints` and the constraint options defined via `ConstraintOptions`.

primer_specificity

Primer specificity is automatically determined during the primer coverage computations but the constraint is only checked when the `primer_specificity` field is available. The specificity of a primer is defined as its ratio of on-target vs total coverage events (including off-target coverage). Low-specificity primers should be excluded as they may not amplify the target region effectively.

primer_length

The length of a primer is defined by its number of bases. Typical primers have lengths between 18 and 22. Longer primers may guarantee higher specificities.

gc_clamp

The GC clamp refers to the presence of GCs at the 3' end of a primer. For the `gc_clamp` constraint, we consider the number of 3' terminal GCs. For example, the primer *actgaaatttcaccg* has a GC clamp of length 3. The presence of a GC clamp is supposed to aid the stability of the polymerase complex. At the same time, long GC clamps should be avoided.

no_runs

Homopolymer runs (e.g. the primer *aaaaa* has a run of 5 A's) may lead to secondary structure formation and unspecific binding and should therefore be avoided.

no_repeats

Dinucleotide repeats (e.g. the primer *tatata* has 3 TA repeats) should be avoided for the same reason a long homopolymer runs.

self_dimerization

Self dimerization refers to a primer that binds to itself rather than to one of the templates. Primers exhibiting self dimers should be avoided as they may prevent the primer from amplifying the templates. Therefore primers with small free energies of dimerization should be avoided.

melting_temp_range

The melting temperature is the temperature at which 50 are in duplex with templates and 50 Hence, primers exhibiting high melting temperatures have high affinities to the templates, while primers with small melting temperatures have small affinities. The melting temperatures of the primers determine the annealing temperature of the PCR, which is why the melting temperatures of the primers should not deviate too much (see `melting_temp_diff`).

melting_temp_diff

The differences between the melting temperatures of primers in a set of primers should not deviate too much as the annealing temperaturte of a PCR should be based on the smallest melting temperature of a primer in the set. If there are other primers in the set exhibiting considerably higher melting temperatures, these primers may bind inspecifically due to the low annealing temperature.

cross_dimerization

When two different primers bind to each each other rather than to the templates, this is called cross dimerization. Cross dimerization should be prevent at all costs because such primers cannot effectively amplify their target templates. Cross dimerizing primers can be excluding primers exhibiting small free energies of cross dimerization.

secondary_structure

When a primer exhibits secondary structure, this may prevent it from binding to the templates. To prevent this, primers with low free energies of secondary structure formation can be excluded.

Note

The following external programs are required for constraint computations:

MELTING (http://www.ebi.ac.uk/biomodels/tools/melting/): Thermodynamic computations (optional) for determining melting temperatures for the constraints `melting_temp_diff` and `melting_temp_range`

OligoArrayAux (http://unafold.rna.albany.edu/OligoArrayAux.php): Thermodynamic computations used for computing `self_dimerization` and `cross_dimerization`. Also required for computing `primer_coverage` when a constraint based on the free energy of annealing is active.

ViennaRNA (http://www.tbi.univie.ac.at/RNA/): Secondary structure predictions used for the constraint `secondary_structure`

The following external programs are required for computing the coverage constraints:

OligoArrayAux (http://unafold.rna.albany.edu/OligoArrayAux.php): Thermodynamic computations used for computing the coverage constraints `annealing_DeltaG`, `primer_efficiency`, and `coverage_model`

See Also

read_settings for reading settings from XML files, write_settings for storing settings as XML files, constraints for accessing constraints, constraintLimits for accessing constraint boundaries, cvg_constraints for accessing coverage constraints, conOptions for accessing constraint options, PCR for accessing the PCR conditions.

Examples

```
# Initializing a new 'ConstraintSettings' object:
constraint.settings <- new("ConstraintSettings")
# Retrieving the constraint settings from a 'DesignSettings' object:
data(Ippolito) # loads a 'DesignSettings' object into 'settings'
constraints(settings)
# Modifying the constraint settings:
constraints(settings)$no_runs["max"] <- 10
constraints(settings) <- constraints(settings)[names(constraints(settings)) != "gc_clamp"]

# Initialize a new 'CoverageConstraints' object:
cvg.constraints <- new("CoverageConstraints")
# Retrieving the coverage constraints from a 'DesignSettings' object:
data(Ippolito) # loads a 'DesignSettings' object into 'settings'
cvg_constraints(settings)
# Modifying the coverage constraints
cvg_constraints(settings)$primer_efficiency["min"] <- 0.001

# Initialize a new 'ConstraintOptions' object:
constraint.options <- new("ConstraintOptions")
# Retrieve the constraint options from a 'DesignSettings' object:
data(Ippolito) # loads a 'DesignSettings' object into 'settings'
conOptions(settings)
# Prevent off-target binding:
conOptions(settings)$allowed_other_binding_ratio <- 0

# Initialize a new 'PCR_Conditions' object:
PCR.conditions <- new("PCR_Conditions")
# Retrieving the PCR conditions from a 'DesignSettings' object:
data(Ippolito) # loads a 'DesignSettings' object into 'settings'
PCR(settings)
# Modifying the PCR conditions:
PCR(settings)$use_taq_polymerase <- FALSE

# Load a settings object
filename <- system.file("extdata", "settings",
                "C_Taq_PCR_high_stringency.xml", package = "openPrimeR")
settings <- read_settings(filename)
# Modify the constraints
constraints(settings)$gc_clamp["min"] <- 0
# Modify the constraint limits for designing primers
constraintLimits(settings)$gc_clamp["max"] <- 6
# Modify the coverage constraints
cvg_constraints(settings)$primer_efficiency["min"] <- 0.001
# Modify the PCR conditions
```

```
PCR(settings)$Na_concentration <- 0.0001
# Modify the constraint options
conOptions(settings)$allowed_mismatches <- 0

# Load some settings
data(Ippolito)
# View the active constraints
constraints(settings)
# Require a minimal GC clamp extent of 0
constraints(settings)$gc_clamp["min"] <- 0
# View available constraints
settings

# Load some settings
data(Ippolito)
# View all active coverage constraints
cvg_constraints(settings)
# Increase the maximal false positive rate to increase the sensitiviity of coverage predictions
cvg_constraints(settings)$coverage_model <- c("max" = 0.1)
# View available coverage constraints:
settings

# Load some settings
data(Ippolito)
# View the active constraint limits
constraintLimits(settings)
# Extend the GC relaxation limit
constraintLimits(settings)$gc_clamp <- c("min" = 0, "max" = 6)
# View available constraints
settings

# Load some settings
data(Ippolito)
# View the active PCR conditions
PCR(settings)
# Evaluate primers with a fixed annealing temperature
PCR(settings)$annealing_temperature <- 50 # celsius
# View available PCR conditions
settings

# Load some settings
data(Ippolito)
# View the active constraint options
conOptions(settings)
# Prevent mismatch binding events
conOptions(settings)$allowed_mismatches <- 0
# View available constraint options
settings
# Use two cores for parallel processing:
parallel_setup(2)
```

shannon.entropy *Shannon Entropy*

Description

Computation of Shannon entropy for an alignment.

Usage

```
shannon.entropy(ali)
```

Arguments

ali	An alignment of primer sequences.

Value

The Shannon entropy for the alignment.

solve.ILP *Solve an ILP*

Description

Constructs and solves an ILP and outputs a list with the reuslts.

Usage

```
## S3 method for class 'ILP'
solve(
  cur.D,
  cur.G,
  cur.settings,
  cur.cvg.matrix,
  time.limit,
  required.cvg,
  primer.df,
  template.df
)
```

Arguments

cur.D	Binary dimerization matrix.
cur.G	Free energy matrix for cross-dimerization.
cur.settings	Current DesignSettings object.
cur.cvg.matrix	Binary coverage matrix.
time.limit	Time limit for solving the ILP in seconds.
required.cvg	The target coverage of the designed primer set.
primer.df	A Primers object.
template.df	A Templates object.
deltaG.cutoff	Cutoff for dimerization free energy.
deltaG.limit	Relaxation limit for free energy cutoff.

Value

List with ILP solution data.

---

split_str_by_index *Split a sequence*

Description

Splits a sequence at a specified positions

Usage

```
split_str_by_index(target, index)
```

Arguments

target	The target string.
index	The position for the split.

Value

List with splitted strings

stats_plot_data *Combination of Filtering Stats.*

Description

Summarizes filtering/relaxation statistics for plotting.

Usage

```
stats_plot_data(stats, stats.relax)
```

Arguments

stats	Statistics of the filtering procedure.
stats.relax	Statistic of the relaxation procedure.

Value

A data frame combinin filtering/relaxation stats.

store.filtering.sets *Writes Filtering Data Sets to Disk.*

Description

Writes Filtering Data Sets to Disk.

Usage

```
store.filtering.sets(
  filtered.df,
  excluded.df,
  results.loc,
  tag = "",
  stat.df = NULL,
  settings = NULL
)
```

Arguments

filtered.df	A filtered Primers set.
excluded.df	A set of Primers that were excluded.
results.loc	The location where to store the data.
tag	A tag for the output files.
stat.df	Data frame with statistics of the filtering procedure.
settings	A DesignSettings object.

Value

No return value, writes output to disk.

`string.list.format` *Format a String List.*

Description

Formats a string list, summarizing values with percentages.

Usage

```
string.list.format(values, order.mode = c("percentage", "value"))
```

Arguments

`values`	The string list to format.
`order.mode`	How the result should be ordered. For "percentage", the strings are ordered by their percentages, while for "value", the strings are ordered by their values.

Value

A formatted string with percentage annotations.

`string.list.format.total`
Format Strings

Description

Changes the representation of the comma-separated string input.

Usage

```
string.list.format.total(values)
```

Arguments

`values`	A comma-separated string with values.

Value

A percentage-formatted representation of the input string.

string.to.IQR *Conversion of Comma-Separated String to IQR String*

Description

Conversion of Comma-Separated String to IQR String

Usage

```
string.to.IQR(string.values)
```

Arguments

string.values	A vector of comma-separated numeric strings.

Value

The IQR corresponding to the input string.

subset.ILP *Subset ILP Constructor*

Description

Constructs an ILP for selecting optimal primer subsets.

Usage

```
## S3 method for class 'ILP'
subset(primer.df, template.df, k)
```

Arguments

primer.df	Primer data frame to be subsetted.
template.df	Template data frame.
k	Required number of primers to be selected.

Details

Here, "optimal" refers to a subset of a certain size that maximizes the coverage.

Value

An ILP for choosing the primer subset of size k with the largest coverage.

TemplatesFunctions *Template Functionalities.*

Description

`adjust_binding_regions` Adjusts the existing annotation of binding regions by specifying a new binding interval relative to the existing binding region.

`assign_binding_regions` Assigns the primer target binding regions to a set of template sequences.

`update_template_cvg` Annotates the template coverage.

`select_regions_by_conservation` Computes Shannon entropy for the defined binding regions and determines the most conserved regions.

Usage

```
update_template_cvg(template.df, primer.df, mode.directionality = NULL)

adjust_binding_regions(template.df, region.fw, region.rev)

assign_binding_regions(
  template.df,
  fw = NULL,
  rev = NULL,
  optimize.region = FALSE,
  primer.length = 20,
  gap.char = "-"
)

select_regions_by_conservation(
  template.df,
  gap.char = "-",
  win.len = 30,
  by.group = TRUE,
  mode.directionality = c("both", "fw", "rev")
)
```

Arguments

`template.df`	An object of class `Templates`.
`primer.df`	An object of class `Primers` containing primers with annotated coverage that are to be used to update the template coverage in `template.df`.
`mode.directionality`	The directionality of primers/templates.
`region.fw`	Interval of new binding regions relative to the forward binding region defined in `template.df`.

region.rev — Interval of new binding regions relative to the reverse binding region defined in template.df

fw — Binding regions for forward primers. Either a numeric interval indicating a uniform binding range relative to the template 5' end or a path to a FASTA file providing binding sequences for every template. If fw is missing, only rev is considered.

rev — Binding regions for reverse primers. Either a numeric interval indicating a uniform binding range relative to the template 3' end or the path to a FASTA file providing binding sequences for every template. If rev is missing, only fw is considered.

optimize.region — If TRUE, the binding regions specified via fw and rev are adjusted such that binding regions that may form secondary structures are avoided. This feature requires ViennaRNA (see notes). If FALSE (the default), the input binding regions are not modified.

primer.length — A numeric scalar providing the probe length that is used for adjusting the primer binding regions when optimize.region is TRUE.

gap.char — The character in the input file representing gaps.

win.len — The extent of the desired primer binding region. This should be smaller than the allowed.region. The default is 30.

by.group — Shall the determination of binding regions be stratified according to the groups defined in template.df. By default, this is set to TRUE.

Details

When modifying binding regions with adjust_binding_regions, new binding intervals can be specified via fw and rev for forward and reverse primers, respectively. The new regions should be provided relative to the existing definition of binding regions in template.df. For specifying the new binding regions, position 0 refers to the first position after the end of the existing binding region. Hence, negative positions relate to regions within the existing binding region, while non-negative values relate to positions outside the defined binding region.

Binding regions are defined using assign_binding_regions, where the arguments fw and rev provide data describing the binding regions of the forward and reverse primers, respectively. To specify binding regions for each template individually, fw and rev should provide the paths to FASTA files. The headers of these FASTA file should match the headers of the loaded template.df and the sequences in the files specified by fw and rev should indicate the target binding regions.

To specify uniform binding regions, fw and rev should be numeric intervals indicating the allowed binding range for primers in the templates. Setting the forward interval to (1,30) indicates that the first 30 bases should be used for forward primers and specifying the reverse interval to (1,30) indicates that the last 30 bases should be used for reverse primer binding.

If optimize.region is TRUE, the input binding region is adjusted such that regions forming secondary structures are avoided.

Value

update_template_cvg returns an object of class Templates with updated coverage columns.

adjust_binding_regions returns a Templates object with updated binding regions.

assign_binding_regions returns an object of class Templates with newly assigned binding regions.

select_regions_by_conservation returns a Templates object with adjusted binding regions. The attribute entropies gives a data frame with positional entropies and the attribute alignments gives the alignments of the templates.

Note

assign_binding_regions requires the program ViennaRNA (https://www.tbi.univie.ac.at/RNA/) for adjusting the binding regions when optimize.region is set to TRUE.

select_regions_by_conservation requires the MAFFT software for multiple alignments (http://mafft.cbrc.jp/alignment/s

Examples

```
# Annotate the coverage of the templates
data(Ippolito)
template.df <- update_template_cvg(template.df, primer.df)
data(Ippolito)
# Extend the binding region by one position
relative.interval <- c(-max(template.df$Allowed_End_fw), 0)
template.df.adj <- adjust_binding_regions(template.df, relative.interval)
# compare old and new annotations:
head(cbind(template.df$Allowed_Start_fw, template.df$Allowed_End_fw))
head(cbind(template.df.adj$Allowed_Start_fw, template.df.adj$Allowed_End_fw))
data(Ippolito)
# Assignment of individual binding regions
l.fasta.file <- system.file("extdata", "IMGT_data", "templates",
     "Homo_sapiens_IGH_functional_leader.fasta", package = "openPrimeR")
template.df.individual <- assign_binding_regions(template.df, l.fasta.file, NULL)
# Assign the first/last 30 bases as forward/reverse binding regions
template.df.uniform <- assign_binding_regions(template.df, c(1,30), c(1,30))
# Optimization of binding regions (requires ViennaRNA)
## Not run: template.df.opti <- assign_binding_regions(template.df, c(1,30), c(1,30),
                     optimize.region = TRUE, primer.length = 20)
## End(Not run)
data(Ippolito)
new.template.df <- select_regions_by_conservation(template.df)
```

---

ungap_sequence *Ungapping of Sequences.*

Description

Removes gaps from the input sequences.

Usage

```
ungap_sequence(seqs, gap.char = "-")
```

Arguments

seqs	The input character vector with sequences
gap.char	The character used to represent gaps.

Value

seqs with gaps removed.

---

unify.leaders *Unification of Leaders*

Description

Unifies individual binding regions for forward and reverse primers.

Usage

```
unify.leaders(l.seq.fw, l.seq.rev, lex.seq, gap.char)
```

Arguments

l.seq.fw	Data frame with binding information for forward primers.
l.seq.rev	Data frame with binding information for reverse primers.
lex.seq	Template data frame.
gap.char	The character for indicating alignment gaps.

Value

Template data frame with annotated binding regions.

---

update.binding.ranges.by.conservation *Updates Binding Region in the Alignment by conservation.*

Description

Updates Binding Region in the Alignment by conservation.

Usage

```
## S3 method for class 'binding.ranges.by.conservation'
update(
  template.df,
  bins,
  entropy.df,
  gap.char = "-",
  win.len = 30,
  direction = c("fw", "rev")
)
```

Arguments

template.df	A Templates object.
bins	A list with DNAbin alignments, one for each group of template sequences.
entropy.df	A data frame with entropy information.
gap.char	The gap character for alignments.
win.len	The desired length of the new binding region.
direction	The direction for which the binding range shall be adjusted.

Value

A Templates object with modified binding regions.

---

update.binding.regions

Update of Binding Regions.

Description

Updates the binding regions in the templates by providing new intervals for forward and reverse binding regions.

Usage

```
## S3 method for class 'binding.regions'
update(template.df, opti.regions)
```

Arguments

template.df	An object of class Templates.
opti.regions	List with new binding intervals. The list can contain the components fw and rev providing numeric vectors of length 2 providing the start and end of the binding regions in the templates, for forward and reverse binding regions, respectively.

Value

A `Templates` object with updated binding regions.

`update.constraint.values` *Update of Primer Constraints.*

Description

Updates the input primer data frame with the computed constraint values.

Usage

```
## S3 method for class 'constraint.values'
update(constraint.df, constraint.values)
```

Arguments

`constraint.df`	Primer data frame.
`constraint.values`	Data frame with computed constraint values.

Value

A primer data frame with updated columns.

`update.cvg.data` *Update Coverage Information.*

Description

Updates the coverage-related columns in the input primer data frame. Does not modify the entries of template-specific coverage columns such as primer efficiency (comma-separated values).

Usage

```
## S3 method for class 'cvg.data'
update(
  filtered.df,
  sel,
  template.df,
  mode = c("on_target", "off_target"),
  active.constraints
)
```

Arguments

filtered.df	Primer data frame.
sel	List with indices of covered templates to be retained, one list with template indices to keep per primer.
template.df	Template data frame.
mode	Either on_target to filter on-target binding events or off_target to filter off-target binding events. The corresponding sel argument should be different.
active.constraints	The active coverage constraints.

Details

Removes all coverage events of templates whose index is not in sel.

Value

A primer data frame with updated coverage information.

---

update.individual.binding.region

Adjustment of Existing Binding Regions for one Direction.

Description

Adjusts the existing annotation of binding regions by specifying an interval relative to the existing binding region.

Usage

```
## S3 method for class 'individual.binding.region'
update(min, max, template.df, mode.directionality)
```

Arguments

min	Position where binding should start.
max	End position of binding.
mode.directionality	Directionality of primers.
Template	data frame.

Details

Position 0 indicates the first position after the existing binding region. Hence, negative positions adjust the binding region towards the existing binding regions and non-negative positionis extend the existing binding region definition aways from the existing target region.

Value

Template data frame with updated binding regions.

`update.opti.results` *Augmentation of Optimized Primer Data.*

Description

Adds melting_temp_diff and cross_dimerization info to optimized sets.

Usage

```
## S3 method for class 'opti.results'
update(primer.df, settings, template.df)
```

Arguments

`primer.df`	A primer data frame.
`settings`	A `DesignSettings` object.

Value

An updated primer data frame.

`update_primer_binding_regions`
Update of Primer Binding Regions.

Description

Updates the relative primer binding sites in the templates when the template binding regions have changed since the last coverage computation.

Usage

```
update_primer_binding_regions(primer.df, template.df, old.template.df)
```

Arguments

`primer.df`	A `Primers` data frame.
`template.df`	Templates with the new binding regions.
`old.template.df`	Templates with the old binding regions.

Value

A `Primers` object with updated relative binding positions.

update_primer_cvg *Updates the Primer Coverage.*

Description

Updates the most important columns in a primer data frame according to the selected coverage definition. Only coverage events with less or equal than the allowed number of mismatches according to the selected coverage definition will be retained.

Usage

```
update_primer_cvg(
  primer.df,
  template.df,
  allowed.mismatches,
  cvg.definition = c("constrained", "basic")
)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.
allowed.mismatches	A numeric giving the maximal number of allowed.mismatches.
cvg.definition	The definition of coverage to be used, either "constrained" or "basic".

Value

A primer data frame with modified coverage information.

validate_primers *Validates a Primers Object.*

Description

Checks whether a Primers object is valid or not.

Usage

```
validate_primers(object)
```

Arguments

object	An input data frame to be checked for being a primer data frame.

Value

TRUE, if the object is valid, FALSE otherwise.

---

validate_templates *Validates a Template Object.*

Description

Checks whether a Templates object is valid or not.

Usage

```
validate_templates(object)
```

Arguments

object	An input data frame to be checked for being a template data frame.

Value

TRUE, if the object is valid, FALSE otherwise.

---

view.cvg.primers *View the Evaluated Primers.*

Description

Creates a formatted primers table.

Usage

```
view.cvg.primers(
  primer.df,
  template.df,
  mode.directionality,
  view.cvg.individual = c("active", "inactive"),
  for.shiny = TRUE
)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.
mode.directionality	The direction of the primers.
view.cvg.individual	Whether information on individual coverage events should be retrained.
for.shiny	Whether the table is intended for Shiny (HTML) or not.

Value

A formatted primer table.

`view.dimer.df` *Formatted dimerization data.*

Description

Format a dimerization data frame for frontend output.

Usage

```
view.dimer.df(dimers, type = c("Self", "Cross"))
```

Arguments

`dimers`	Dimerization data frame.
`type`	Type of dimerization.

Value

A data frame whose columns are formatted in a user-readable way.

`view.input.primers` *View the Input Primers.*

Description

Creates a formatted primers table.

Usage

```
view.input.primers(primer.df, mode.directionality, for.shiny = TRUE)
```

Arguments

`primer.df`	A `Primers` object.
`mode.directionality`	The direction of the primers.
`for.shiny`	Whether output is intended for Shiny.

Value

A formatted primer table.

view.primers *View the Evaluated Primers.*

Description

Creates a formatted primers table.

Usage

```
view.primers(primer.df, template.df)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.

Value

A formatted primer table.

view.primers.report *View the Evaluated Primers in the Report.*

Description

Creates a formatted primers table for the report PDF.

Usage

```
view.primers.report(primer.df, template.df)
```

Arguments

primer.df	A Primers object.
template.df	A Templates object.

Value

A formatted primer table.

`visualize.all.results` *Visualization of Design Results.*

Description

Visualizes all results from designing primers.

Usage

```
visualize.all.results(
  sample,
  filtering.results.loc,
  opti.results.loc,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  settings,
  mode.directionality,
  used.settings,
  required.cvg
)
```

Arguments

`sample`	Identifier of the design run.
`filtering.results.loc`	Location of filtering results.
`opti.results.loc`	Location of optimization results.
`primer_conc`	Primer concentration.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris ion concentration.
`settings`	The `DesignSettings` object.
`mode.directionality`	Strand direction for which primers were designed.
`used.settings`	A list with the used settings for optimization (fields "fw" and "rev").
`required.cvg`	The required coverage.
`template_conc`	Template concentration.

Value

Writes visualizations to files in.

`visualize.filtering.results` *Visualization of Filtering Results.*

Description

Visualizes the filtering results.

Usage

```
visualize.filtering.results(
  sample,
  results.loc,
  mode.directionality,
  excluded.df,
  template.df,
  filtered.df,
  filtered.stats,
  stats.relax,
  primer_conc,
  na_salt_conc,
  mg_salt_conc,
  k_salt_conc,
  tris_salt_conc,
  settings,
  required.cvg
)
```

Arguments

`sample`	Primer design run identifier.
`results.loc`	Location where the filtering results are stored.
`mode.directionality`	Design direction.
`excluded.df`	Data frame with excluded primers.
`template.df`	Template data frame.
`filtered.df`	Primer data frame containing the primers that passed the constraints.
`stats.relax`	Filtering statistics after relaxation.
`primer_conc`	Primer concentration.
`na_salt_conc`	Sodium ion concentration.
`mg_salt_conc`	Magensium ion concentration.
`k_salt_conc`	Potassium ion concentration.
`tris_salt_conc`	Tris ion concentration.

settings	A DesignSettings object.
required.cvg	The required coverage.
template_conc	Template concentration.

Value

Write-out of filtering results.

---

were.constraints.relaxed *Check for Relaxation*

Description

Determines whether constraints where relaxed or not.

Usage

```
were.constraints.relaxed(used.constraints, input.constraints)
```

Arguments

used.constraints	The constraints that were used during the optimization.
input.constraints	The user-specified constraints.

Value

If input.constraints was relaxed TRUE is returned, otherwise FALSE.

---

write.out.primer.info *Write Out Optimization Data*

Description

Writes out all data relating to the optimization of primers.

Usage

```
write.out.primer.info(
  opti.results.loc,
  optimal.primers.data,
  mode.directionality,
  settings,
  sample.name,
  template.df,
  max.degen
)
```

Arguments

opti.results.loc	Folder where optimization data reside.
optimal.primers.data	List with optimization results.
mode.directionality	Direction of primers.
settings	Settings used in the optimization procedure. List containing fw, rev settings.
sample.name	Name of template sample.
template.df	Template data frame.
max.degen	Maximal degeneracy of primers.

Value

Write-out of primer information to opti.results.loc.

---

xmlToChar *Conversion of XML to Character.*

Description

Converts an XML object to a character string.

Usage

```
xmlToChar(xml)
```

Arguments

xml	An xml object to be converted to character.

Value

A character vector.

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