Package ‘openPrimeR’

May 30, 2024

Title Mulitplex PCR Primer Design and Analysis

Version 1.26.0

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 7.3.0

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), RCColorBrewer (>= 1.1-2), DECIIPHER (>= 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
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openPrimeR-package

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`.

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Package 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.

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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, please 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 belong-
ing to a specific group. By default, total.percentages is FALSE such 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.

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

getcvgstatsprimer returns a list with the following entries. cvg_per_nbr_mismatches con-
tains 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.

getcvgstats 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 identity 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])

## 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

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

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

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

```r
## 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"

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
**check_cvg_constraints**

**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**

```r
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"
```

**Description**

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

**Usage**

```r
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.

---

**Description**

Creates a PDF report for comparing multiple primers.

**Usage**

```r
## 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).

Description

Creation of a PDF report.

Usage

```r
## 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 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).


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	iller.template.df
tiller.settings

constraints(tiller.settings)
### Description

**filterLimits**

Getter for Filtering Constraint Limits.

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**

```r
filterLimits(x)
```

---

### 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**

```r
filters(x)
```

---

### Arguments

- **x**
  
  A `DesignSettings` object.

### Value

- Gets the list of filtering limits.

- Gets the list of filtering constraints.
**get_cvg_stats,list-method**

*Coverage Statistics for Multiple Primer Sets.*

**Description**

Retrieve statistics on covered templates for multiple primer sets.

**Usage**

```r
## 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.
**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 sequences 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 number 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; otherwise 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.
**Input**

**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 `Primer` 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**

```r
# 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 multiple 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)

---

**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

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>template.df</td>
<td>An object of class Templates.</td>
</tr>
<tr>
<td>fname</td>
<td>The path to the output file.</td>
</tr>
<tr>
<td>ftype</td>
<td>A character vector giving the type of the file. This can either be &quot;FASTA&quot;</td>
</tr>
<tr>
<td></td>
<td>or &quot;CSV&quot; (default: &quot;FASTA&quot;).</td>
</tr>
<tr>
<td>primer.df</td>
<td>An object of class Primers.</td>
</tr>
<tr>
<td>settings</td>
<td>A DesignSettings object to be stored to disk.</td>
</tr>
<tr>
<td>primers</td>
<td>To create a report for a single primer set, please provide an evaluated</td>
</tr>
<tr>
<td></td>
<td>Primers object. For creating a report comparing multiple primer sets, please</td>
</tr>
<tr>
<td></td>
<td>provide a list of Primers objects.</td>
</tr>
<tr>
<td>templates</td>
<td>If primers is a Primers object, templates should be a Templates object.</td>
</tr>
<tr>
<td></td>
<td>If primers is a list of Primers objects, templates should be a list of</td>
</tr>
<tr>
<td></td>
<td>Templates objects of the same length as primers.</td>
</tr>
<tr>
<td>sample.name</td>
<td>An identifier for your analysis. By default (NULL), the sample identifier is</td>
</tr>
<tr>
<td></td>
<td>selected from the Run column of the input templates.</td>
</tr>
<tr>
<td>used.settings</td>
<td>A named list (with fields fw and rev) containing the relaxed settings for</td>
</tr>
<tr>
<td></td>
<td>designing forward/reverse primers. By default (NULL), the relaxed settings</td>
</tr>
<tr>
<td></td>
<td>are not shown in the report.</td>
</tr>
</tbody>
</table>
... 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)
set.sizes <- 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)

---

### Plots

#### Plotting Functions.

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>plot_cvg_vs_set_size</td>
<td>Plots the coverage ratios of the input primer sets against the size of the sets.</td>
</tr>
<tr>
<td>plot_penalty_vs_set_size</td>
<td>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.</td>
</tr>
<tr>
<td>plot_primer_subsets</td>
<td>Visualizes the coverage of optimized primer subsets.</td>
</tr>
<tr>
<td>plot_primer</td>
<td>Visualizes the binding positions of every primer relative to the target binding region in the corresponding template sequences.</td>
</tr>
<tr>
<td>plot_template_cvg</td>
<td>Creates a bar plot visualizing the covered templates.</td>
</tr>
<tr>
<td>plot_primer_cvg</td>
<td>Shows which groups of templates are covered by individual primers.</td>
</tr>
<tr>
<td>plot_constraint</td>
<td>Shows the distribution of the primer properties. The current constraint settings are indicated with dashed lines.</td>
</tr>
<tr>
<td>plot_constraint_fulfillment</td>
<td>Visualizes which primers pass the constraints and which primers break the constraints</td>
</tr>
<tr>
<td>plot_cvg_constraints</td>
<td>Plots the distribution of the coverage constraint values.</td>
</tr>
<tr>
<td>plot_constraint_deviation</td>
<td>Plots the deviation of primer properties from the target ranges.</td>
</tr>
<tr>
<td>plot_primer_binding_regions</td>
<td>Visualizes the number of binding events of the primers with respect to the allowed binding regions in the templates.</td>
</tr>
<tr>
<td>plot_conservation</td>
<td>Plots the template sequence conservation (range [0,1]) according to the Shannon entropy of the sequences.</td>
</tr>
</tbody>
</table>
### Usage

```r
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
)


```r
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.
Plots

**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_conseration` requires the MAFFT software for multiple alignments (http://mafft.cbrc.jp/alignment/software/).

**Examples**

```r
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_conseration(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_conseration(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 multiple 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**

```r
## 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**

```r
## 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_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**

```r
## 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

---

**Description**

Plots a box plot of deviations of primer properties from the target ranges.

**Usage**

```r
## 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
**Description**

Plots the percentage of primers fulfilling the specified constraints for multiple primer sets.

**Usage**

```r
## 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 constraints 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`. 
Overview of Constraint Fulfillment.

Description

Plots an overview of which primers passed the filtering constraints and which primers did not.

Usage

```r
## 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 constraints 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 for Comparing Primer Coverage Constraints.

Description

Plot for Comparing Primer Coverage Constraints.
Usage

```r
## 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.

Description

Plots a histogram of coverage constraint values.

Usage

```r
## 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 of Primer Binding Regions for Multiple Sets.

Description

Plots the primer binding regions for every primer set.

Usage

```r
## 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 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.

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.
**plot_primer_cvg,Primers,Templates-method**

*Plot Individual Primer Coverage.*

**Description**

Shows which templates are covered by individual primers.

**Usage**

```r
## 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_template_cvg,list,list-method**

*Templates Coverage for Multiple Primer Sets.*

**Description**

Plots the coverage of multiple primer sets.

**Usage**

```r
## S4 method for signature 'list,list'
plot_template_cvg(primers, templates, per.mismatch, highlight.set = 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.
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.

---

### Bar Plot of Template Coverage.

**Description**

Creates a bar plot showing the coverage for every group of template sequences.

**Usage**

```r
## 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.
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,
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 covered template sequences. If the target coverage ratio cannot be reached, the constraint settings are relaxed according to 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 optialgo 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()
PrimerEval

Primer Evaluation.

Description

check_constraints Determines whether a set of primers fulfills the constraints on the properties of the primers.

cHECK_restrictions_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 default 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


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
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

```r
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

```r
## Not run:
# Open the tutorial
if (interactive()) {
  runTutorial()
}

## End(Not run)
```
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

```r
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

```r
## 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)

## Settings

### 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

canstraints(x)

## S4 method for signature 'DesignSettings'
canstraints(x)

## S4 method for signature 'AbstractConstraintSettings'
canstraints(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)

canstraints(x) <- value

## S4 replacement method for signature 'DesignSettings,list'
canstraints(x) <- value

## S4 replacement method for signature 'AbstractConstraintSettings,list'
canstraints(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'

```r
PCR(x) <- value
conOptions(x) <- value
```

## S4 replacement method for signature 'DesignSettings'

```r
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 constraint 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 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 \([\text{kcal/mol}]\) 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 \([\text{kcal/mol}]\) 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 maximum 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 automatically 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 `actgaattccaccg` 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 `aaaaaa` 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 temperature 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/](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](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/](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:


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

```r
# 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 sensitivity of coverage predictions
coverage_model <- c("max" = 0.1)
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
```r
# 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)
```

**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**

```r
# Adjust binding regions
adjust_binding_regions(template.df, region.fw, region.rev)

# Assign binding regions
assign_binding_regions(
  template.df,
  fw = NULL,
  rev = NULL,
  optimize.region = FALSE,
  primer.length = 20,
  gap.char = "-"
)

# Select regions by conservation
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 uni-
            form 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 uni-
            form 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 re-
            gions 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/software).

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)
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