

Package ‘barcodePrimer’

January 6, 2020

Type Package

Title A package to randomly generate barcoded primer set combinations without primer dimers

Version 0.1.0

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Description This tiny package aims at the barcoded primer sets for amplicon sequencing. Based on the report of primer set in Thermo Fisher Scientific, this package will parse the result, and then randomly generate barcoded primer set.

Use four spaces when indenting paragraphs within the Description.

URL <https://github.com/cam315/barcodePrimer>

License GPL(>=3)

Encoding UTF-8

LazyData true

RemoteType github

RemoteHost <https://api.github.com>

RemoteRepo barcodePrimer

RemoteUsername cam315

RemoteRef master

RemoteSha d896edbf522941667b70751ddbb005c3cd19030a

GithubRepo barcodePrimer

GithubUsername cam315

GithubRef master

GithubSHA1 d896edbf522941667b70751ddbb005c3cd19030a

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dropDimer	<i>find the primer identifiers with self dimers</i>
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Description

This function will parse the primer checking report and give out the self-dimer primer identifiers.

Usage

```
dropDimer(report, ndimer)
```

Arguments

report	the report file which was generated by the webtool from Thermo Fisher.
ndimer	argument to specify threshold at which self-dimer primer will be filtered out. If you specify 2, all primer with 2 or more than 2 self dimers will be found out.

Details

this function will find out the primer identifiers with two or three self-dimer in the barcoded primers.
This function accept ndimer given in numeric values like 2 and 3, or in character 'two' or 'three'.

Value

return a vector containing the self-dimer primer identifiers.

Author(s)

Sizhong Yang <yanglzu@163.com>

Examples

```
data(report)
selfdimers = dropDimer(report, ndimer = 3)

## remove the selfdimers from the result

data(PrimerLabels)

wcomb = reparser(report)
tcomb = primerCombi(PrimerLabels)

vcombs = slim(tcomb, wcomb)
moreprimers = pickmore(combi = vcombs, n = 120)
dim(moreprimers)

## further remove the primers with selfdimers
```

```
toDrop = apply(moreprimers, 1, function(x) any(match(x, selfdimers, nomatch = 0) > 0))
outprimers = moreprimers[!toDrop,]
dim(outprimers)
```

pickmore

Pick up the combination of non-dimer primer sets.

Description

In some case, the unique barcoded primers are less than the amount of samples. More primer sets can be generated by this function according to the number you specified. In this case, some primer (either forward or reverse) may occur more than one time, which may lead to difficulty in demultiplexing the sequencing data. For technical reasons, this specified number should be less than 2 times of the unique barcode primer you have.

Usage

```
pickmore(combi, n)
```

Arguments

combi	argument to specify the unique primer set which have been filtered by removing primer dimer combinations.
n	numeric, a integer to specify the number of primer sets. For technical reasons, n should be less than 2 times of the number of unique pairs of initial barcoded primers.

Value

The function will return a data frame of two columns. The 1st and 2nd columns show the identifiers of forward primer and reverse primer, respectively.

Author(s)

Sizhong Yang <yanglzu@163.com>

Examples

```
data(report)
data(PrimerLabels)
wcomb = reparser(report)
tcomb = primerCombi(PrimerLabels)

combs = slim(tcomb, wcomb)
moreprimers = pickmore(combi = combs, n = 120)
dim(moreprimers)
```

pickunique	<i>randomly pick up the combination of primer sets without reported primer dimers.</i>
------------	--

Description

This function will randomly pick up the unique combination of primer sets. Unique means all the forward and reverse barcoded primer will be unique (used only once). Therefore, the resultant primer sets will be in the same amount as the input PrimerLabels file.

Usage

```
pickunique(combi)
```

Arguments

combi	argument to specify the unique primer set which have been filtered by removing primer dimer combinations.
-------	---

Details

The file to assigned to argument combi will be generated by slim function in this package. That is a random combination of barcoded primer sets within which the primer dimer sets were removed.

Value

The function will return a data frame of two columns. The 1st and 2nd columns show the identifiers of forward primer and reverse primer, respectively. If combinations more than the amount of input primerlabels are expected, please use the function 'pickmore'.

Author(s)

Sizhong Yang <yanglzu@163.com>

Examples

```
data(report)
data(PrimerLabels)
wcomb = reparser(report)
tcomb = primerCombi(PrimerLabels)

combs = slim(tcomb, wcomb)
uprimers = pickunique(combi = combs)
dim(uprimers)
```

primerCombi	<i>generate the theoretical combinations of barcoded primers</i>
-------------	--

Description

This function will generate all possible combinations of barcoded primer sets.

Usage

```
primerCombi(primerfile)
```

Arguments

primerfile arguments to specify the primer identifier file.

Value

The file will generate a data frame of two columns, each row gives a primer combination.

Author(s)

Sizhong Yang <yanglzu@163.com>

Examples

```
data(PrimerLabels)
tcomb = primerCombi(PrimerLabels)
dim(tcomb)
```

PrimerLabels	<i>Example data showing the valid primer lable table</i>
--------------	--

Description

A data frame containing 2 columns of primer identifiers (Note, not the sequence of barcode or barcoded primer itself). The first and second columns represent forward and reverse primers, respectively. The forward primer labels must end with 'forward' or 'for' (case insensitive), connected other info with '_' or '-', e.g. 'Uni515-10-For'. The reverse follows similar format, ending with 'reverse' or 'rev'.

Usage

```
data("PrimerLabels")
```

Format

A data frame with 2 columns and 96 rows, each row represent the identifier of barcoded primer.

Forward a character vector

Reverse a character vector

Details

The identifiers in this example come from a custom dataset, amongst 'Uni515' represent universal bacterial primer starting at position 515, and 'Uni806' denotes that the universal bacterial primer with position 806.

Examples

```
data(PrimerLabels)
names(PrimerLabels)
dim(PrimerLabels)
```

reparser	<i>Parse the report of barcoded-primer to get primer combinations with cross dimers.</i>
----------	--

Description

This function will parse the primer-check report generated by webtool 'Multiple Primer Analyzer' from Thermo Fisher Scientific, and extract the primer combinations with cross dimers.

Usage

```
reparser(repfile)
```

Arguments

repfile	argument specify the report file. It can be a file handle (name) or a report file which have been read into.
---------	--

Value

this function will return a two-column data frame, each row will a be primer combination with cross dimers.

Author(s)

Sizhong Yang <yanglzu@163.com>

Examples

```
data(report)

wcomb = reparser(repfile = report)
dim(wcomb)
head(wcomb)
```

report	<i>The check report on barcoded primers which were generated by Thermo Fisher Scientific.</i>
--------	---

Description

The report is about the barcoded primer sets,

Usage

```
data("report")
```

Format

A dataset with 26332 rows.

x a numeric vector

y a numeric vector

Details

This dataset were evaluated by the webtool 'Multiple Primer Analyzer' from Thermo Fisher Scientific. The 'parameters for detection of primer dimers' was set to '1', value 1 is maximum sensitive detection. This file include both 'Self-Dimers' and 'Cross Primer Dimers', indicating the primer dimers which may hamper the binning efficiency on template during PCR.

Source

see the entire dataset at <https://github.com/cam315/barcodePrimer>

Examples

```
data(report)
length(report)
```

slim	<i>filter the primer set combinations.</i>
------	--

Description

function to remove primer combinations from the theoretical combinations.

Usage

```
slim(x, y)
```

Arguments

x Character, the file of all theoretical combination of primer sets.

y Character, the file of the primer combinations with theoretical dimers.

Details

In this function, the primer combinations with cross primer dimer will be removed from the theoretical combinations of primer set.

Value

This function will return a two-column data frame, giving the valid primer combinations.

Author(s)

Sizhong Yang <yanglzu@163.com>

Examples

```
data(report)
data(PrimerLabels)

## generate all possible combinations for the primers

tcomb = primerCombi(PrimerLabels)

## find out all cross dimer combinations

wcomb = reparser(repfile = report)

## get primer combination by removing the cross dimers

vcomb = slim(x = tcomb, y = wcomb)
dim(vcomb)
head(vcomb)
```

wrapped

A wrapped function to directly generate primer sets according to input files

Description

This function will generate primer combinations based on the files of primer check report by Multiple Primer Analyzer in Thermo Fisher Scientific, primer list and the specified number of primer sets

Usage

```
wrapped(repfile, primerfile, n)
```

Arguments

repfile	Arguments to specify the file name of primer check result from Multiple Primer Analyzer in Thermo Fisher Scientific. The user can either specify the file handle (name) or variable containing the file.
primerfile	Arguments to specify the two-column file of primer identifiers. This file is requested to follow the format of primeExample.

n Numeric, integer to specify the number of primer sets you expected. For technical reasons, n should be less than 2 times of the number of unique pairs of initial barcoded primers.

Value

The function will return a data frame of two columns. The 1st and 2nd columns show the identifiers of forward primer and reverse primer, respectively. Each row is a combination of primer set which has passed the primer check process, and (potentially/most probably) work in PCR amplification.

Author(s)

Sizhong Yang <yanglzu@163.com>

Examples

```
data(report)
data(PrimerLabels)

result <- wrapped(repfile = report, primerfile = PrimerLabels, n=120)

dim(result)
head(result)
```

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