

# Package ‘DECIPHER’

October 7, 2014

**Type** Package

**Title** Database Enabled Code for Ideal Probe Hybridization Employing R

**Version** 1.10.1

**Date** 2013-10-07

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**biocViews** Clustering, Genetics, Sequencing, Infrastructure,DataImport, Visualization, Microarray, QualityControl

**Description** A toolset that assist in the design of hybridization probes.

**Depends** R (>= 2.13.0), Biostrings (>= 2.31.9), RSQLite (>= 0.9),stats, parallel

**Imports** methods, IRanges, XVector

**LinkingTo** Biostrings, RSQLite, IRanges, XVector

**License** GPL-3

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DECIPHER-package	<i>Database Enabled Code for Ideal Probe Hybridization Employing R</i>
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## Description

Database Enabled Code for Ideal Probe Hybridization Employing R (DECIPHER) is a software toolset that can be used for deciphering and managing biological sequences efficiently using the R statistical programming language. The program is designed to be used with non-destructive workflows that guide the user through the process of importing, maintaining, analyzing, manipulating, and exporting a massive amount of sequences. Some functionality of the program is provided on-line through web tools. DECIPHER is an ongoing project at the University of Wisconsin Madison and is freely available for download.

## Details

Package:	DECIPHER
Type:	Package
Depends:	R (>= 2.13.0), Biostrings (>= 2.31.9), RSQLite (>= 0.9), stats, parallel
Imports:	methods, IRanges, XVector
LinkingTo:	Biostrings, RSQLite, IRanges, XVector
License:	GPL-3
LazyLoad:	yes

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IdConsensus	Create Consensus Sequences by Groups
IdentifyByRank	Identify By Taxonomic Rank
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MaskAlignment	Masks Highly Variable Regions of An Alignment
MODELS	Available Models of DNA Evolution
NNLS	Sequential Coordinate-wise Algorithm for the Non-negative Least Squares Problem
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Seqs2DB	Add Sequences from Text File to Database
TerminalChar	Determine the Number of Terminal Characters
TileSeqs	Form a Set of Tiles for Each Group of Sequences

**Author(s)**

Erik Wright

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Add2DB

*Add Data To A Database*

---

## Description

Adds a `data.frame` to a database table by `row.names`.

## Usage

```
Add2DB(myData,  
        dbFile,  
        tblName = "DNA",  
        clause = "",  
        verbose = TRUE)
```

## Arguments

<code>myData</code>	Data frame containing information to be added to the <code>dbFile</code> .
<code>dbFile</code>	A SQLite connection object or a character string specifying the path to the database file.
<code>tblName</code>	Character string specifying the table in which to add the data.
<code>clause</code>	An optional character string to append to the query as a clause.
<code>verbose</code>	Logical indicating whether to display each query as it is sent to the database.

## Details

Data contained in `myData` will be added to the `tblName` by its respective `row.names`.

## Value

Returns TRUE if the data was added successfully, or FALSE otherwise.

## Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

## See Also

[Seqs2DB](#), [SearchDB](#), [BrowseDB](#)

**Examples**

```
# Create a sequence database
gen <- system.file("extdata", "Bacteria_175seqs.gen", package="DECIPHER")
dbConn <- dbConnect(SQLite(), ":memory:")
Seqs2DB(gen, "GenBank", dbConn, "Bacteria")

# Identify the sequence lengths
l <- IdLengths(dbConn)

# Add lengths to the database
Add2DB(l, dbConn)

# View the added lengths
BrowseDB(dbConn)
dbDisconnect(dbConn)
```

---

AlignDB

*Aligns Two Sets of Aligned Sequences In A Sequence Database*

---

**Description**

Merges the two separate sequence alignments in a database. The aligned sequences must have separate identifiers in the same table or be located in different database tables.

**Usage**

```
AlignDB(dbFile,
        tblName = "DNA",
        identifier = "",
        type = "DNAStringSet",
        add2tbl = "DNA",
        batchSize = 10000,
        perfectMatch = 6,
        misMatch = 0,
        gapOpening = -9,
        gapExtension = -3,
        terminalGap = -2,
        substitutionMatrix = "BLOSUM62",
        processors = NULL,
        verbose = TRUE)
```

**Arguments**

dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table(s) where the sequences are located. If two tblNames are provided then the sequences in both tables will be aligned.

identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" then all identifiers are selected. If two identifiers are provided then the set of sequences matching each identifier will be aligned.
type	The type of XStringSet being processed. This should be (an unambiguous abbreviation of) one of "AAStringSet", "DNAStringSet", or "RNAStringSet".
add2tbl	Character string specifying the table name in which to add the aligned sequences.
batchSize	Integer specifying the number of sequences to process at a time.
perfectMatch	Numeric giving the reward for aligning two matching nucleotides in the alignment.
misMatch	Numeric giving the cost for aligning two mismatched nucleotides in the alignment.
gapOpening	Numeric giving the cost for opening a gap in the alignment.
gapExtension	Numeric giving the cost for extending an open gap in the alignment.
terminalGap	Numeric giving the cost for allowing leading and trailing gaps in the alignment. Either two numbers, the first for leading gaps and the second for trailing gaps, or a single number for both.
substitutionMatrix	Name of the amino acid substitution matrix to use in alignment. Must be one of: "BLOSUM45", "BLOSUM50", "BLOSUM62", "BLOSUM80", "BLOSUM100", "PAM30", "PAM40", "PAM70", "PAM120", "PAM250". Only applicable for AAStringSet inputs.
processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.

### Details

Sometimes it is useful to align two large sets of sequences, where each set of sequences is already aligned but the two sets are not aligned to each other. This function first builds a profile of each sequence set in increments of batchSize so that the entire sequence set is not required to fit in memory. Next the two profiles are aligned using dynamic programming. Finally, the new alignment is applied to all the sequences as they are incrementally added to the add2tbl.

### Value

Returns the number of newly aligned sequences added to the database.

### Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

### See Also

[AlignProfiles](#), [AlignSeqs](#), [AlignTranslation](#)

**Examples**

```

gen <- system.file("extdata", "Bacteria_175seqs.gen", package="DECIPHER")
fas <- system.file("extdata", "Bacteria_175seqs.fas", package="DECIPHER")

# Align two tables and place result into a third
dbConn <- dbConnect(SQLite(), ":memory:")
Seqs2DB(gen, "GenBank", dbConn, "Seqs1", tblName="Set1")
Seqs2DB(fas, "FASTA", dbConn, "Seqs2", tblName="Set2")
AlignDB(dbConn, tblName=c("Set1", "Set2"), add2tbl="AlignedSets")
l <- IdLengths(dbConn, "AlignedSets", add2tbl=TRUE)
BrowseDB(dbConn, tblName="AlignedSets") # all sequences have the same width
dbDisconnect(dbConn)

# Align two identifiers and place the result in the same table
dbConn <- dbConnect(SQLite(), ":memory:")
Seqs2DB(gen, "GenBank", dbConn, "Seqs1")
Seqs2DB(fas, "FASTA", dbConn, "Seqs2")
AlignDB(dbConn, identifier=c("Seqs1", "Seqs2"))
l <- IdLengths(dbConn, add2tbl=TRUE)
BrowseDB(dbConn) # all sequences have the same width
dbDisconnect(dbConn)

```

---

AlignProfiles

*Aligns Two Sets of Aligned Sequences*


---

**Description**

Aligns two sets of one or more aligned sequences by first generating representative profiles, then aligning the profiles with dynamic programming, and finally merging the two aligned sequence sets.

**Usage**

```

AlignProfiles(pattern,
              subject,
              p.weight = 1,
              s.weight = 1,
              perfectMatch = 6,
              misMatch = 0,
              gapOpening = -9,
              gapExtension = -3,
              terminalGap = -2,
              restrict = -1000,
              anchor = 0.7,
              substitutionMatrix = "BLOSUM62",
              processors = NULL)

```

**Arguments**

pattern	An AStringSet, DNStringSet, or RNStringSet object of aligned sequences to use as the pattern.
subject	A XStringSet object of aligned sequences to use as the subject. Must match the type of the pattern.
p.weight	A numeric vector of weights for each sequence in the pattern to use in generating a profile, or a single number implying equal weights.
s.weight	A numeric vector of weights for each sequence in the subject to use in generating a profile, or a single number implying equal weights.
perfectMatch	Numeric giving the reward for aligning two matching nucleotides in the alignment.
misMatch	Numeric giving the cost for aligning two mismatched nucleotides in the alignment.
gapOpening	Numeric giving the cost for opening a gap in the alignment.
gapExtension	Numeric giving the cost for extending an open gap in the alignment.
terminalGap	Numeric giving the cost for allowing leading and trailing gaps in the alignment. Either two numbers, the first for leading gaps and the second for trailing gaps, or a single number for both.
restrict	Numeric specifying the lowest relative score to consider when aligning. The default (-1000) will align most inputs that can reasonably be globally aligned without any loss in accuracy. Input sequences with high similarity could be more restricted (e.g., -500), whereas a pattern and subject with little overlap should be less restricted (e.g., -10000). (See details section below.)
anchor	Numeric giving the fraction of sequences with identical k-mers required to become an anchor point, or NA to not use anchors. (See details section below.)
substitutionMatrix	Name of the amino acid substitution matrix to use in alignment. Must be one of: "BLOSUM45", "BLOSUM50", "BLOSUM62", "BLOSUM80", "BLOSUM100", "PAM30", "PAM40", "PAM70", "PAM120", "PAM250". Only applicable for AStringSet inputs.
processors	The number of processors to use, or NULL (the default) for all available processors.

**Details**

Profiles are aligned using dynamic programming, a variation of the Needleman-Wunsch algorithm for global alignment. This method works by filling in a matrix of the possible "alignment space" by considering all matches, insertions, and deletions between two sequence profiles. The highest scoring alignment is then used to add gaps to each of the input sequence sets. The default input parameters have been optimized to align amino acid and RNA sequences using the structural benchmarks. Careful consideration should be given to the choice of substitutionMatrix (for AStringSet inputs) and sequence weightings so that they reflect the divergence between input sequences.

The dynamic programming method requires order  $N \times M$  time and memory space where  $N$  and  $M$  are the width of the pattern and subject. Therefore heuristics can be useful to improve performance on

long input sequences. The `restrict` parameter can be used to dynamically constrain the possible “alignment space” to only paths that will likely include the final alignment, which in the best case can improve the speed from quadratic time to linear time. The degree of restriction is critically important, and if the sequences are not necessarily mostly overlapping then `restrict` should be relaxed (lower than the default). For example, if aligning a short profile to a long profile then `restrict` should be set to `-Inf`.

The argument `anchor` can be used to split the global alignment into multiple sub-alignments. This can greatly decrease the memory requirement for long sequences when appropriate anchor points can be found. Anchors are 15-mer (for DNA/RNA) or 7-mer (for AA) subsequences that are shared between between at least anchor fraction of pattern(s) and subject(s). Anchored ranges are extended along the length of each sequence in a manner designed to split the alignment into sub-alignments that can be separately solved. For most input sequences `anchor` has little or no effect on accuracy, but anchoring can be disabled by setting `anchor=NA`.

### Value

An `XStringSet` of aligned sequences.

### Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

### References

Needleman S., Wunsch, C. (1970). A general method applicable to the search for similarities in the amino acid sequence of two proteins. *Journal of Molecular Biology*, **48(3)**, 443-453.

### See Also

[AlignDB](#), [AlignSeqs](#), [AlignTranslation](#)

### Examples

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
dna1 <- SearchDB(db, remove="common", limit=100) # the first 100 sequences
dna2 <- SearchDB(db, remove="common", limit="100,100") # the rest
alignedDNA <- AlignProfiles(dna1, dna2)
BrowseSequences(alignedDNA, highlight=1)
```

---

AlignSeqs

*Aligns A Set of Unaligned Sequences*

---

### Description

Performs profile-to-profile alignment of multiple unaligned sequences following a guide tree.

**Usage**

```
AlignSeqs(myXStringSet,
          guideTree = NULL,
          orient = FALSE,
          processors = NULL,
          verbose = TRUE,
          ...)
```

**Arguments**

myXStringSet	An AAStringSet, DNASTringSet, or RNASTringSet object of unaligned sequences.
guideTree	Either NULL or a data.frame giving the ordered tree structure in which to align profiles. If NULL then a guide tree will be constructed.
orient	Logical specifying whether some sequences may need to be reoriented before alignment. If TRUE, an attempt to determine the best orientation (reverse and/or complement) will be performed with sequences reoriented as necessary to match the orientation of the longest sequence. Not applicable for an AAStringSet input.
processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.
...	Further arguments to be passed directly to <a href="#">AlignProfiles</a> , including perfectMatch, mismatch, gapOpening, gapExtension, terminalGap, restrict, and anchor.

**Details**

The profile-to-profile method aligns a sequence set by merging profiles along a guide tree until all sequences are aligned. If `guideTree=NULL`, an initial UPGMA guide tree is constructed based on a distance matrix of shared k-mers. A second guide tree is built based on the initial alignment, and the alignment is refined using this tree. If a `guideTree` is provided then sequences are only aligned once. The `guideTree` should be provided in the output given by `IdClusters` with ascending levels of cutoff.

**Value**

An XStringSet of aligned sequences.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[AlignDB](#), [AlignProfiles](#), [AlignTranslation](#), [IdClusters](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
dna <- SearchDB(db, limit=10, remove="all")
alignedDNA <- AlignSeqs(dna)
BrowseSequences(alignedDNA, highlight=1)
```

AlignTranslation

*Aligns Sequences By Their Amino Acid Translation***Description**

Performs alignment of a set of DNA or RNA sequences by aligning their corresponding amino acid sequences.

**Usage**

```
AlignTranslation(myXStringSet,
                 sense = "+",
                 direction = "5 to 3",
                 readingFrame = NA,
                 asAAStringSet = FALSE,
                 ...)
```

**Arguments**

myXStringSet	A DNASTringSet or RNASTringSet object of unaligned sequences.
sense	Single character specifying sense of the input sequences, either the positive ("+") coding strand or negative ("-") non-coding strand.
direction	Direction of the input sequences, either "5 to 3" or "3 to 5".
readingFrame	Numeric vector giving a single reading frame for all of the sequences, or an individual reading frame for each sequence in myXStringSet. The readingFrame can be either 1, 2, 3 to begin translating on the first, second, and third nucleotide position, or NA (the default) to guess the reading frame. (See details section below.)
asAAStringSet	Logical specifying whether to return the aligned translation as an AAStringSet rather than an XStringSet of the input type.
...	Further arguments to be passed directly to <a href="#">AlignSeqs</a> , including perfectMatch, misMatch, gapOpening, gapExtension, terminalGap, restrict, anchor, doNotAlign, guideTree, processors, and verbose.

**Details**

Alignment of proteins is often more accurate than alignment of their coding nucleic acid sequences. This function aligns the input nucleic acid sequences via aligning their translated amino acid sequence. First, the input sequences are translated according to the specified sense, direction, and readingFrame. The resulting amino acid sequences are aligned with the function `AlignSeqs`, and this alignment is reverse translated into the original sequence type, sense, and direction.

If the readingFrame is NA (the default) then an attempt is made to guess the reading frame of each sequence based on the number of stop codons in the translated amino acids. For each sequence, the first reading frame will be chosen (either 1, 2, or 3) that has one or zero stop codons in the last position. If the number of stop codons is inconclusive for a sequence then the reading frame will default to 1. The entire length of each sequence is translated in spite of any stop codons identified. Note that this method is only constructive in circumstances where there is a substantially long coding sequence with at most a single stop codon expected at the final position, and therefore it is preferable to specify the reading frame of each sequence if it is known.

**Value**

An XStringSet matching the input type.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[AlignDB](#), [AlignProfiles](#), [AlignSeqs](#)

**Examples**

```
# First three sequences translate to MFITP*, last sequence is MF-TP*
rna <- RNAStringSet(c("AUGUUCAUCACCCCUAA", "AUGUUCAUAACUCCUUGA",
  "AUGUUCAUUACACCGUAG", "AUGUUUACCCCAUAA"))
RNA <- AlignSeqs(rna)
BrowseSequences(RNA) # incorrect gap position

RNA <- AlignTranslation(rna)
BrowseSequences(RNA) # correct gap position
```

---

Array2Matrix

*Creates a Matrix Representation of a Microarray*

---

**Description**

Converts the output of `DesignArray` into the sparse matrix format used by NNLS.

**Usage**

```
Array2Matrix(probes,  
             verbose = TRUE)
```

**Arguments**

probes	A set of microarray probes in the format output by DesignArray.
verbose	Logical indicating whether to display progress.

**Details**

A microarray can be represented by a matrix of hybridization efficiencies, where the rows represent each of the probes and the columns represent each the possible templates. This matrix is sparse since microarray probes are designed to only target a small subset of the possible templates.

**Value**

A list specifying the hybridization efficiency of each probe to its potential templates.

i	Element's row index in the sparse matrix.
j	Element's column index in the sparse matrix.
x	Non-zero elements' values representing hybridization efficiencies.
dimnames	A list of two components: the names of each probe, and the names of each template.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**References**

Coming Soon!

**See Also**

[DesignArray](#), [NNLS](#)

**Examples**

```
fas <- system.file("extdata", "Bacteria_175seqs.fas", package="DECIPHER")  
dna <- readDNASTringSet(fas)  
names(dna) <- 1:length(dna)  
probes <- DesignArray(dna)  
A <- Array2Matrix(probes)
```

---

BrowseDB

*View A Database Table In A Web Browser*

---

### Description

Opens an html file in a web browser to show the contents of a table in a database.

### Usage

```
BrowseDB(dbFile,  
         htmlFile = paste(tempdir(), "/db.html", sep = ""),  
         tblName = "DNA",  
         identifier = "",  
         limit = -1,  
         orderBy = "row_names",  
         maxChars = 50,  
         clause="")
```

### Arguments

dbFile	A SQLite connection object or a character string specifying the path to the database file.
htmlFile	Character string giving the location where the html file should be written.
tblName	Character string specifying the table to view.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" then all identifiers are selected.
limit	Number of results to display. The default (-1) does not limit the number of results.
orderBy	Character string giving the column name for sorting the results. Defaults to the order of entries in the database. Optionally can be followed by " ASC" or " DESC" to specify ascending (the default) or descending order.
maxChars	Maximum number of characters to display in each column.
clause	An optional character string to append to the query as a clause.

### Value

Creates a table containing all the fields of the database table and opens it in the web browser for easy viewing.

Returns htmlFile if the html file was written successfully.

### Note

If viewing a table containing sequences, the sequences are purposefully not shown in the output.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[BrowseSequences](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
BrowseDB(db)
```

---

BrowseSequences

*View Sequences In A Web Browser*

---

**Description**

Opens an html file in a web browser to show the sequences in an XStringSet.

**Usage**

```
BrowseSequences(myXStringSet,
                htmlFile = paste(tempdir(), "/myXStringSet.html", sep = ""),
                colorPatterns = TRUE,
                highlight = 0,
                patterns = c("-", alphabet(myXStringSet, baseOnly=TRUE)),
                colors = substring(rainbow(length(patterns),
                                          v=0.8, start=0.9, end=0.7), 1, 7),
                colWidth = Inf,
                ...)
```

**Arguments**

myXStringSet	A XStringSet object of sequences.
htmlFile	Character string giving the location where the html file should be written.
colorPatterns	Logical specifying whether to color matched patterns, or an integer vector providing pairs of start and stop boundaries for coloring.
highlight	Numeric specifying which sequence in the set to use for comparison or 0 to color all sequences (default).
patterns	Character vector containing regular expressions to be colored in the XStringSet. Regular expressions are searched sequentially with multiple matches allowed, even within other previously matched patterns. (See details section below.)
colors	Character vector providing the color for each of the matched patterns. Typically a character vector with elements of 7 characters, “#” followed by the red, blue, green values in hexadecimal (after rescaling to 0 ... 255). Positions with background color have white font.

<code>colWidth</code>	Integer giving the maximum number of nucleotides wide the display can be before starting a new page. Must be a multiple of 20 (e.g., 100), or <code>Inf</code> (the default) to display all the sequences in one set of rows.
<code>...</code>	Additional arguments to adjust the appearance of the consensus sequence at the base of the display. Passed directly to <code>ConsensusSequence</code> for an <code>AAStringSet</code> , <code>DNAStrngSet</code> , or <code>RNAStringSet</code> , or to <code>consensusString</code> for a <code>BStringSet</code> .

### Details

Some web browsers cannot quickly display a large amount colored text, so it is recommended to use `color = FALSE` when viewing a large `XStringSet`.

Patterns are not matched across column breaks, so multi-character patterns should be carefully considered when `colWidth` is less than the maximum sequence length. Patterns are matched sequentially in the order provided, so it is feasible to use nested patterns such as `c("ACCTG", "CC")`. In this case the "CC" could be colored differently inside the previously colored "ACCTG". Note that patterns overlapping the boundaries of a previously matched pattern will not be matched. For example, "ACCTG" would not be matched if `patterns=c("CC", "ACCTG")`.

Column positions identical to the highlighted sequence are replaced with a "." character. Patterns are not matched in "." regions.

### Value

Creates an html file containing sequence data and opens it in a web browser for easy viewing. The viewer has the sequence name on the left, position legend on the top, cumulative number of nucleotides on the right, and consensus sequence on the bottom.

Returns `htmlFile` if the html file was written successfully.

### Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

### See Also

[BrowseDB](#), [ConsensusSequence](#)

### Examples

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
dna <- SearchDB(db)
BrowseSequences(dna, colWidth=100, highlight=1)

# color bases in alternating groups with a different color scheme
BrowseSequences(dna[1:5],
  colorPatterns=seq(1, width(dna)[1], 10),
  patterns=c("A", "C", "G", "T", "-"),
  colors=c("#1E90FF", "#32CD32", "#9400D3", "#000000", "#EE3300"))

# color all restriction sites
data(RESTRICTION_ENZYMES)
```

```

sites <- RESTRICTION_ENZYMES
sites <- sites[order(nchar(sites))] # match shorter sites first
# convert all restriction sites into regular expressions
for (degeneracy in names(IUPAC_CODE_MAP)[5:15]) {
  sites <- gsub(degeneracy,
    paste("[", degeneracy, "|",
      paste(strsplit(IUPAC_CODE_MAP[degeneracy], "")[[1]],
        collapse="|"),
      "]",
      sep=""),
    sites)
}
dna <- SearchDB(db, remove="all") # unaligned sequences
BrowseSequences(dna, patterns=sites)

```

---

## CalculateEfficiencyArray

*Predicts the Hybridization Efficiency of Probe/Target Sequence Pairs*

---

### Description

Calculates the Gibbs free energy and hybridization efficiency of probe/target pairs at varying concentrations of the denaturant formamide.

### Usage

```

CalculateEfficiencyArray(probe,
  target,
  FA = 0,
  dGini = 1.96,
  Po = 10^-2.0021,
  m = 0.1731,
  temp = 42,
  deltaGrules = NULL)

```

### Arguments

probe	A DNASTringSet object or character vector with pairwise-aligned probe sequences in 5' to 3' orientation.
target	A DNASTringSet object or character vector with pairwise-aligned target sequences in 5' to 3' orientation.
FA	A vector of one or more formamide concentrations (as percent v/v).
dGini	The initiation free energy. The default is 1.96 [kcal/mol].
Po	The effective probe concentration.
m	The m-value defining the linear relationship of denaturation in the presence of formamide.

temp	Equilibrium temperature in degrees Celsius.
deltaGrules	Free energy rules for all possible base pairings in quadruplets. If NULL, defaults to the parameters obtained using NimbleGen microarrays and a Linear Free Energy Model developed by Yilmaz <i>et al.</i>

### Details

This function calculates the free energy and hybridization efficiency (HE) for a given formamide concentration ([FA]) using the linear free energy model given by:

$$HE = Po * exp[-(dG_0 + m * FA)/RT] / (1 + Po * exp[-(dG_0 + m * FA)/RT])$$

Probe and target input sequences must be entered in pairwise alignment, such as that given by pairwiseAlignment. Only "A", "C", "G", "T", and "-" characters are permitted in the probe sequence.

If deltaGrules is NULL then the rules defined in data(deltaGrules) are used.

### Value

A matrix with the predicted Gibbs free energy (dG) and hybridization efficiency (HE) at each concentration of formamide ([FA]).

### Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

### References

Yilmaz LS, Loy A, Wright ES, Wagner M, Noguera DR (2012) Modeling Formamide Denaturation of Probe-Target Hybrids for Improved Microarray Probe Design in Microbial Diagnostics. PLoS ONE 7(8): e43862. doi:10.1371/journal.pone.0043862.

### See Also

[deltaGrules](#)

### Examples

```
probes <- c("AAAAACGGGGAGCGGGGGATACTG", "AAAAACTCAACCCGAGGAGCGGGGG")
targets <- c("CAACCCGGGGAGCGGGGGATACTG", "TCGGGCTCAACCCGAGGAGCGGGGG")
result <- CalculateEfficiencyArray(probes, targets, FA=0:40)
dG0 <- result[, "dG_0"]
HE0 <- result[, "HybEff_0"]
plot(result[1, 1:40], xlab="[FA]", ylab="HE", main="Probe/Target # 1", type="l")
```

---

`CalculateEfficiencyFISH`*Predicts Thermodynamic Parameters of Probe/Target Sequence Pairs*

---

**Description**

Calculates the Gibbs free energy, formamide melt point, and hybridization efficiency of probe/target (DNA/RNA) pairs.

**Usage**

```
CalculateEfficiencyFISH(probe,  
                        target,  
                        temp,  
                        P,  
                        ions,  
                        FA,  
                        batchSize = 1000)
```

**Arguments**

probe	A DNASTringSet object or character vector with unaligned probe sequences in 5' to 3' orientation.
target	A DNASTringSet object, RNASTringSet, or character vector with unaligned target or non-target sequences in 5' to 3' orientation. The DNA base Thymine will be treated the same as Uracil.
temp	Numeric specifying the hybridization temperature, typically 46 degrees Celsius.
P	Numeric giving the molar concentration of probes during hybridization.
ions	Numeric giving the molar sodium equivalent ionic concentration. Values may range between 0.01M and 1M. Note that salt correction is not available for thermodynamic rules of RNA/RNA interactions, which were determined at 1 molar concentration.
FA	Numeric concentration (as percent v/v) of the denaturant formamide in the hybridization buffer.
batchSize	Integer specifying the number of probes to simulate hybridization per batch. See the Description section below.

**Details**

Hybridization efficiency of pairwise probe/target (DNA/RNA) pairs is simulated *in silico*. Gibbs free energies are obtained from system calls to `OligoArrayAux`, which must be properly installed (see the Notes section below). Probe/target pairs are sent to `OligoArrayAux` in batches of `batchSize`, which prevents systems calls from being too many characters.

**Value**

A matrix of predicted hybridization efficiency (HybEff), formamide melt point (Fam), and free energy (ddG1 and dG1) for each probe/target pair.

**Note**

The program OligoArrayAux (<http://mfold.rna.albany.edu/?q=DINAMelt/OligoArrayAux>) must be installed in a location accessible by the system. For example, the following code should print the installed OligoArrayAux version when executed from the R console:

```
system("hybrid-min -V")
```

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**References**

Coming Soon!

**See Also**

[DesignProbes](#), [TileSeqs](#)

**Examples**

```
probe <- c("GGGCTTTCACATCAGACTTAAGAAACC", "CCCCACGCTTTCGCGCC")
target <- reverseComplement(DNAStringSet(probe))
# not run (must have OligoArrayAux installed first):
#CalculateEfficiencyFISH(probe, target, temp=46, P=250e-9, ions=1, FA=35)
```

---

CalculateEfficiencyPCR

*Predicts Amplification Efficiency of Primer Sequences*

---

**Description**

Calculates the amplification efficiency of primers from their hybridization efficiency and elongation efficiency at the target site.

**Usage**

```
CalculateEfficiencyPCR(primer,
                      target,
                      temp,
                      P,
                      ions,
                      batchSize = 1000,
```

```

    taqEfficiency = TRUE,
    maxDistance = 0.4,
    maxGaps = 2,
    processors = NULL)

```

### Arguments

primer	A DNASTringSet object or character vector with unaligned primer sequences in 5' to 3' orientation.
target	A DNASTringSet object or character vector with unaligned target or non-target sequences in 5' to 3' orientation.
temp	Numeric specifying the annealing temperature used in the PCR reaction.
P	Numeric giving the molar concentration of primers in the reaction.
ions	Numeric giving the molar sodium equivalent ionic concentration. Values may range between 0.01M and 1M.
batchSize	Integer specifying the number of primers to simulate hybridization per batch. See the Description section below.
taqEfficiency	Logical determining whether to make use of elongation efficiency and maxDistance to increase predictive accuracy for <i>Taq</i> DNA Polymerase amplifying primers with mismatches near the 3' terminus. Note that this should be set to FALSE if using a high-fidelity polymerase with 3' to 5' exonuclease activity.
maxDistance	Numeric specifying the maximal fraction of mismatched base pairings on a rolling basis beginning from the 3' end of the primer. Only used if taqEfficiency is TRUE.
maxGaps	Integer specifying the maximum number of insertions or deletions (indels) in the primer/target alignment. Only used if taqEfficiency is TRUE.
processors	The number of processors to use, or NULL (the default) for all available processors.

### Details

Amplification of pairwise primer/target pairs is simulated *in silico*. A complex model of hybridization is employed that takes into account the side reactions resulting in probe-folding, target-folding, and primer-dimer formation. The resulting hybridization efficiency is multiplied by the elongation efficiency to predict the overall efficiency of amplification.

Free energy is obtained from system calls to OligoArrayAux, which must be properly installed (see the Notes section below). Primer/target pairs are sent to OligoArrayAux in batches of batchSize, which prevents systems calls from being too many characters.

### Value

A vector of predicted efficiencies for amplifying each primer/target pair of sequences.

**Note**

The program OligoArrayAux (<http://mfold.rna.albany.edu/?q=DINAMelt/OligoArrayAux>) must be installed in a location accessible by the system. For example, the following code should print the installed OligoArrayAux version when executed from the R console:

```
system("hybrid-min -V")
```

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**References**

ES Wright et al. (2013) "Exploiting Extension Bias in PCR to Improve Primer Specificity in Ensembles of Nearly Identical DNA Templates." *Environmental Microbiology*, doi:10.1111/1462-2920.12259.

**See Also**

[DesignPrimers](#), [TileSeqs](#)

**Examples**

```
primers <- c("AAAAACGGGGAGCGGGGG", "AAAAACTCAACCCGAGGAGCGCGT")
targets <- reverseComplement(DNAStringSet(primers))
# not run (must have OligoArrayAux installed first):
#CalculateEfficiencyPCR(primers, targets, temp=75, P=4e-7, ions=.225)
```

---

ConsensusSequence      *Create A Consensus Sequence*

---

**Description**

Forms a consensus sequence representing a set of sequences.

**Usage**

```
ConsensusSequence(myXStringSet,
                  threshold = 0.05,
                  ambiguity = TRUE,
                  noConsensusChar = "+",
                  minInformation = 0.75,
                  ignoreNonBases = FALSE,
                  includeTerminalGaps = FALSE,
                  verbose = TRUE)
```

**Arguments**

<code>myXStringSet</code>	An <code>AAStringSet</code> , <code>DNAStrngSet</code> , or <code>RNAStringSet</code> object of aligned sequences.
<code>threshold</code>	Maximum fraction of sequence information that may be lost in forming the consensus.
<code>ambiguity</code>	Logical specifying whether to consider ambiguity as split between their respective nucleotides. Degeneracy codes are specified in the <code>IUPAC_CODE_MAP</code> .
<code>noConsensusChar</code>	Single character from the sequence's alphabet giving the base to use when there is no consensus in a position.
<code>minInformation</code>	Minimum fraction of information required to form consensus in each position.
<code>ignoreNonBases</code>	Logical specifying whether to count gap ("-") or mask ("+") characters towards the consensus.
<code>includeTerminalGaps</code>	Logical specifying whether or not to include terminal gaps ("-") characters on each end of the sequence) into the formation of consensus.
<code>verbose</code>	Logical indicating whether to print the elapsed time upon completion.

**Details**

Two key parameters control the degree of consensus. The default `threshold` (0.05) indicates that at least 95% of sequence information will be represented by the consensus sequence. The default `minInformation` (0.75) specifies that at least 75% of sequences must contain the information in the consensus, otherwise the `noConsensusChar` is used.

If `ambiguity` = `TRUE` (the default) then degeneracy codes are split between their respective bases according to the `IUPAC_CODE_MAP`, or `AMINO_ACID_CODE` for `AAStringSets`. For example, an "R" in a `DNAStrngSet` would count as half an "A" and half a "G". If `ambiguity` = `FALSE` then degeneracy codes are not considered in forming the consensus. If `includeNonBases` = `TRUE` (the default) then gap ("-") and mask ("+") characters are counted towards the consensus, otherwise they are omitted from calculation of the consensus. For an `AAStringSet` input, the lack of degeneracy codes generally results in "X" in positions with mismatches, unless the `threshold` is set higher than 0.05.

**Value**

An `XStringSet` matching the input type with a single consensus sequence.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[IdConsensus](#), [Seqs2DB](#)

**Examples**

```
dna <- DNASTringSet(c("ANGCT-", "-ACCT-"))
ConsensusSequence(dna)
# returns "ANSCT-"
```

---

CreateChimeras	<i>Creates Artificial Chimeras</i>
----------------	------------------------------------

---

**Description**

Creates artificial random chimeras from a set of sequences.

**Usage**

```
CreateChimeras(myDNASTringSet,
               numChimeras = 10,
               numParts = 2,
               minLength = 80,
               maxLength = Inf,
               minChimericRegionLength = 30,
               randomLengths = TRUE,
               includeParents = TRUE,
               processors = NULL,
               verbose = TRUE)
```

**Arguments**

myDNASTringSet	A DNASTringSet object with aligned sequences.
numChimeras	Number of chimeras desired.
numParts	Number of chimeric parts from which to form a single chimeric sequence.
minLength	Minimum length of the complete chimeric sequence.
maxLength	Maximum length of the complete chimeric sequence.
minChimericRegionLength	Minimum length of the chimeric region of each sequence part.
randomLengths	Logical specifying whether to create random length chimeras in addition to random breakpoints.
includeParents	Whether to include the parents of each chimera in the output.
processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.

**Details**

Forms a set of random chimeras from the input set of (typically good quality) sequences. The chimeras are created by merging random sequences at random breakpoints. These chimeras can be used for testing the accuracy of the [FindChimeras](#) or other chimera finding functions.

**Value**

A DNAStrngSet object containing chimeras. The names of the chimeras are specified as "parent #1 name [chimeric region] (distance from parent to chimera), ...".

If includeParents = TRUE then the parents of the the chimeras are included at the end of the result. The parents are made to be the same length as the chimera if randomLengths = TRUE. The names of the parents are specified as "parent #1 name [region] (distance to parent #2, ...)".

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[FindChimeras](#), [Seqs2DB](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
dna <- SearchDB(db)
chims <- CreateChimeras(dna)
BrowseSequences(chims)
```

---

DB2Seqs

*Export Database Sequences to a FASTA or FASTQ File*

---

**Description**

Exports a database containing sequences to a FASTA or FASTQ formatted file of sequence records.

**Usage**

```
DB2Seqs(file,
        dbFile,
        tblName = "DNA",
        identifier = "",
        type = "BStringSet",
        limit = -1,
        replaceChar = "-",
        nameBy = "description",
        orderBy = "row_names",
        removeGaps = "none",
        append = FALSE,
        width = 80,
        chunkSize = 1e5,
        clause = "",
        verbose = TRUE)
```

**Arguments**

file	Character string giving the location where the file should be written.
dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table in which to extract the data.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" then all identifiers are selected.
type	The type of XStringSet (sequences) to export to a FASTA formatted file or QualityScaledXStringSet to export to a FASTQ formatted file. This should be (an unambiguous abbreviation of) one of "DNAStrngSet", "RNAStrngSet", "AAStrngSet", "BStringSet", "QualityScaledDNAStrngSet", "QualityScaledRNAStrngSet", "QualityScaledAAStrngSet", or "QualityScaledBStringSet". (See details section below.)
limit	Number of results to display. The default (-1) does not limit the number of results.
replaceChar	Optional character used to replace any characters of the sequence that are not present in the XStringSet's alphabet. Not applicable if type=="BStringSet". (See details section below.)
nameBy	Character string giving the column name(s) for identifying each sequence record. If more than one column name is provided, the information in each column is concatenated, separated by pairs of colons ("::"), in the order specified.
orderBy	Character string giving the column name for sorting the results. Defaults to the order of entries in the database. Optionally can be followed by " ASC" or " DESC" to specify ascending (the default) or descending order.
removeGaps	Determines how gaps are removed in the sequences. This should be (an unambiguous abbreviation of) one of "none", "all" or "common".
append	Logical indicating whether to append the output to the existing file.
width	Integer specifying the maximum number of characters per line of sequence. Not applicable when exporting to a FASTQ formatted file.
chunkSize	Number of lines of the file to write at a time. Cannot be less than the total number of sequences if removeGaps is "common".
clause	An optional character string to append to the query as a clause.
verbose	Logical indicating whether to display status.

**Details**

Sequences are exported into either a FASTA or FASTQ file as determined by the type of sequences. If type is an XStringSet then sequences are exported to FASTA format. Quality information for QualityScaledXStringSets are interpreted as PredQuality scores before export to FASTQ format.

If type is "BStringSet" (the default) then sequences are exported to a FASTA file exactly the same as they were when imported. If type is "DNAStrngSet" then all U's are converted to T's before export, and vice-versa if type is "RNAStrngSet". All remaining characters not in the XStringSet's alphabet are converted to replaceChar.

**Value**

Writes a FASTA or FASTQ formatted file containing the sequence records in the database.

Returns the number of sequence records written to the file.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
tf <- tempfile()
DB2Seqs(tf, db, limit=10)
file.show(tf)
unlink(tf)
```

---

deltaGrules

*Free Energy of Hybridization of Probe/Target Quadruplets*

---

**Description**

The 8D array works with four adjacent base pairs of the probe and target sequence at a time. Each dimension has five elements defining the residue at that position ("A", "C", "G", "T", or "-"). The array contains the standard Gibbs free energy change of probe binding (dG, [kcal/mol]) for every quadruple base pairing.

**Usage**

```
data(deltaGrules)
```

**Format**

The format is: num [1:5, 1:5, 1:5, 1:5, 1:5, 1:5, 1:5, 1:5] -0.141 0 0 0 0 ... - attr(\*, "dimnames")=List of 8 ..\$ : chr [1:5] "A" "C" "G" "T" ... ..\$ : chr [1:5] "A" "C" "G" "T" ... ..\$ : chr [1:5] "A" "C" "G" "T" ... ..\$ : chr [1:5] "A" "C" "G" "T" ... ..\$ : chr [1:5] "A" "C" "G" "T" ... ..\$ : chr [1:5] "A" "C" "G" "T" ... ..\$ : chr [1:5] "A" "C" "G" "T" ... ..\$ : chr [1:5] "A" "C" "G" "T" ...

**Details**

The first four dimensions correspond to the 4 probe positions from 5' to 3'. The fifth to eighth dimensions correspond to the 4 positions from 5' to 3' of the target sequence.

**Source**

Data obtained using NimbleGen microarrays and a Linear Free Energy Model developed by Yilmaz *et al.*

## References

Yilmaz LS, Loy A, Wright ES, Wagner M, Noguera DR (2012) Modeling Formamide Denaturation of Probe-Target Hybrids for Improved Microarray Probe Design in Microbial Diagnostics. PLoS ONE 7(8): e43862. doi:10.1371/journal.pone.0043862.

## Examples

```
data(deltaGrules)
# dG of probe = AGCT / target = A-CT pairing
deltaGrules["A", "G", "C", "T", "A", "-", "C", "T"]
```

---

DesignArray

*Designs a set of DNA Microarray Probes for Detecting Sequences*

---

## Description

Chooses the set of microarray probes maximizing sensitivity and specificity to each target consensus sequence.

## Usage

```
DesignArray(myDNAStringSet,
            maxProbeLength = 24,
            minProbeLength = 20,
            maxPermutations = 4,
            numRecordedMismatches = 500,
            numProbes = 10,
            start = 1,
            end = NULL,
            maxOverlap = 5,
            hybridizationFormamide = 10,
            minMeltingFormamide = 15,
            maxMeltingFormamide = 20,
            minScore = -1e+12,
            processors = NULL,
            verbose = TRUE)
```

## Arguments

- `myDNAStringSet` A DNAStringSet object of aligned consensus sequences.
- `maxProbeLength` The maximum length of probes, not including the poly-T spacer. Ideally less than 27 nucleotides.
- `minProbeLength` The minimum length of probes, not including the poly-T spacer. Ideally more than 18 nucleotides.

maxPermutations	The maximum number of probe permutations required to represent a target site. For example, if a target site has an 'N' then 4 probes are required because probes cannot be ambiguous. Typically fewer permutations are preferable because this requires less space on the microarray and simplifies interpretation of the results.
numRecordedMismatches	The maximum number of recorded potential cross-hybridizations for any target site.
numProbes	The target number of probes on the microarray per input consensus sequence.
start	Integer specifying the starting position in the alignment where potential forward primer target sites begin. Preferably a position that is included in most sequences in the alignment.
end	Integer specifying the ending position in the alignment where potential reverse primer target sites end. Preferably a position that is included in most sequences in the alignment.
maxOverlap	Maximum overlap in nucleotides between target sites on the sequence.
hybridizationFormamide	The formamide concentration (% , vol/vol) used in hybridization at 42 degrees Celsius. Note that this concentration is used to approximate hybridization efficiency of cross-amplifications.
minMeltingFormamide	The minimum melting point formamide concentration (% , vol/vol) of the designed probes. The melting point is defined as the concentration where half of the template is bound to probe.
maxMeltingFormamide	The maximum melting point formamide concentration (% , vol/vol) of the designed probes. Must be greater than the minMeltingFormamide.
minScore	The minimum score of designed probes before exclusion. A greater minScore will accelerate the code because more target sites will be excluded from consideration. However, if the minScore is too high it will prevent any target sites from being recorded.
processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.

### Details

The algorithm begins by determining the optimal length of probes required to meet the input constraints while maximizing sensitivity to the target consensus sequence at the specified hybridization formamide concentration. This set of potential target sites is then scored based on the possibility of cross-hybridizing to the other non-target sequences. The set of probes is returned with the minimum possibility of cross-hybridizing is chosen to represent each consensus sequence.

### Value

A data.frame with the optimal set of probes matching the specified constraints. Each row lists the probe's target sequence (name), start position, length in nucleotides, start and end position

in the sequence alignment, number of permutations, score, melt point in percent formamide at 42 degrees Celsius, hybridization efficiency (hyb\_eff), target site, and probe(s). Probes are designed such that the stringency is determined by the equilibrium hybridization conditions and not subsequent washing steps.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**References**

ES Wright et al. (2013) Identification of Bacterial and Archaeal Communities From Source to Tap. Water Research Foundation, Denver, CO.

**See Also**

[Array2Matrix](#), [NLS](#)

**Examples**

```
fas <- system.file("extdata", "Bacteria_175seqs.fas", package="DECIPHER")
dna <- readDNASTringSet(fas)
names(dna) <- 1:length(dna)
probes <- DesignArray(dna)
probes[1,]
```

---

DesignPrimers

*Designs PCR Primers Targeting a Specific Group of Sequences*

---

**Description**

Assists in the design of primer sets targeting a specific group of sequences while minimizing the potential to cross-amplify other groups of sequences.

**Usage**

```
DesignPrimers(tiles,
               identifier = "",
               start = 1,
               end = NULL,
               minLength = 17,
               maxLength = 26,
               maxPermutations = 4,
               minCoverage = 0.9,
               minGroupCoverage = 0.2,
               annealingTemp = 64,
               P = 4e-07,
               monovalent = 0.07,
```

```

divalent = 0.003,
dNTPs = 8e-04,
minEfficiency = 0.8,
worstScore = -Inf,
numPrimerSets = 0,
minProductSize = 75,
maxProductSize = 1200,
maxSearchSize = 1500,
batchSize = 1000,
maxDistance = 0.4,
primerDimer = 1e-07,
ragged5Prime = TRUE,
taqEfficiency = TRUE,
induceMismatch = FALSE,
processors = NULL,
verbose = TRUE)

```

### Arguments

tiles	A set of tiles representing each group of sequences, as in the format created by the function TileSeqs.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. Determines the target group(s) for which primers will be designed. If "" then all identifiers are selected.
start	Integer specifying the starting position in the alignment where potential forward primer target sites begin. Preferably a position that is included in most sequences in the alignment.
end	Integer specifying the ending position in the alignment where potential reverse primer target sites end. Preferably a position that is included in most sequences in the alignment.
minLength	Integer providing the minimum length of primers to consider in the design.
maxLength	Integer providing the maximum length of primers to consider in the design, which must be less than or equal to the maxLength of tiles.
maxPermutations	Integer providing the maximum number of permutations considered as part of a forward or reverse primer set.
minCoverage	Numeric giving the minimum fraction of the target group's sequences that must be covered with the primer set.
minGroupCoverage	Numeric giving the minimum fraction of the target group that must have sequence information (not terminal gaps) in the region covered by the primer set.
annealingTemp	Numeric indicating the desired annealing temperature that will be used in the PCR experiment.
P	Numeric giving the molar concentration of primers in the reaction.
monovalent	The molar concentration of monovalent ([Na] and [K]) ions in solution that will be used to determine a sodium equivalent concentration.

divalent	The molar concentration of divalent ([Mg]) ions in solution that will be used to determine a sodium equivalent concentration.
dNTPs	Numeric giving the molar concentration of free nucleotides added to the solution that will be used to determine a sodium equivalent concentration.
minEfficiency	Numeric giving the minimum efficiency of hybridization desired for the primer set. Note that an efficiency of 99
worstScore	Numeric specifying the score cutoff to remove target sites from consideration. For example, a worstScore of -5 will remove all primer sets scoring below -5, although this may eventually result in no primer sets meeting the design criteria.
numPrimerSets	Integer giving the optimal number of primer sets (forward and reverse primer sets) to design. If set to zero then all possible forward and reverse primers are returned, but the primer sets minimizing potential cross-amplifications are not chosen.
minProductSize	Integer giving the minimum number of nucleotides desired in the PCR product.
maxProductSize	Integer giving the maximum number of nucleotides desired in the PCR product.
maxSearchSize	Integer giving the maximum number of nucleotides to search for false priming upstream and downstream of the expected binding site.
batchSize	Integer specifying the number of primers to simulate hybridization per batch that is passed to CalculateEfficiencyPCR.
maxDistance	Numeric specifying the maximal fraction of mismatched base pairings on a rolling basis beginning from the 3' end of the primer.
primerDimer	Numeric giving the maximum amplification efficiency of primer-dimer products.
ragged5Prime	Logical specifying whether the 5' end or 3' end of primer permutations targeting the same site should be varying lengths.
taqEfficiency	Logical determining whether to make use of elongation efficiency and maxDistance to increase predictive accuracy for <i>Taq</i> DNA Polymerase amplifying primers with mismatches near the 3' terminus. Note that this should be set to FALSE if using a high-fidelity polymerase with 3' to 5' exonuclease activity.
induceMismatch	Logical or integer specifying whether to induce a mismatch in the primer with the template DNA. If TRUE then a mismatch is induced at the 6th primer position. If an integer value is provided between 2 and 6 then a mismatch is induced in that primer position, where the 3'-end is defined as position 1.
processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.

### Details

Primers are designed for use with *Taq* DNA Polymerase to maximize sensitivity and specificity for the target group of sequences. The design makes use of *Taq*'s bias against certain 3' terminal mismatch types in order to increase specificity further than can be achieved with hybridization efficiency alone.

Primers are designed from a set of tiles to target each identifier while minimizing affinity for all other tiled groups. Arguments provide constraints that ensure the designed primer sets meet the specified criteria as well as being optimized for the particular experimental conditions. A search is conducted through all tiles in the same alignment position to estimate the chance of cross-amplification with a non-target group.

If numPrimers is greater than or equal to one then the set of forward and reverse primers that minimizes potential false positive overlap is returned. This will also initiate a thorough search through all target sites upstream and downstream of the expected binding sites to ensure that the primers do not bind to nearby positions. Lowering the maxSearchSize will speed up the thorough search at the expense of potentially missing an unexpected target site. The number of possible primer sets assessed is increased with the size of numPrimers.

### Value

A different data.frame will be returned depending on number of primer sets requested. If no primer sets are required then columns contain the forward and reverse primers for every possible position scored by their potential to amplify other identified groups. If one or more primer sets are requested then columns contain information for the optimal set of forward and reverse primers that could be used in combination to give the fewest potential cross-amplifications.

### Note

The program OligoArrayAux (<http://mfold.rna.albany.edu/?q=DINAMelt/OligoArrayAux>) must be installed in a location accessible by the system. For example, the following code should print the installed OligoArrayAux version when executed from the R console:

```
system("hybrid-min -V")
```

To install OligoArrayAux from the downloaded source folder on Unix-like platforms, open the shell (or Terminal on Mac OS) and type:

```
cd oligoarrayaux # change directory to the correct folder name
./configure
make
sudo make install
```

### Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

### References

ES Wright et al. (2013) "Exploiting Extension Bias in PCR to Improve Primer Specificity in Ensembles of Nearly Identical DNA Templates." Environmental Microbiology, doi:10.1111/1462-2920.12259.

### See Also

[CalculateEfficiencyPCR](#), [TileSeqs](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
# not run (must have OligoArrayAux installed first):
#tiles <- TileSeqs(db, identifier=c("Acinetobacter","Pseudomonas"))
#primers <- DesignPrimers(tiles, identifier="Acinetobacter", start=280, end=420,
# minProductSize=50, numPrimerSets=1)
```

DesignProbes

*Designs FISH Probes Targeting a Specific Group of Sequences***Description**

Assists in the design of single or dual probes targeting a specific group of sequences while minimizing the potential to cross-hybridize with other groups of sequences.

**Usage**

```
DesignProbes(tiles,
             identifier = "",
             start = 1,
             end = NULL,
             minLength = 17,
             maxLength = 26,
             maxPermutations = 4,
             minCoverage = 0.9,
             minGroupCoverage = 0.2,
             hybTemp = 46,
             P = 2.5e-07,
             Na = 1,
             FA = 35,
             minEfficiency = 0.5,
             worstScore = -Inf,
             numProbeSets = 0,
             batchSize = 1000,
             target = "SSU",
             verbose = TRUE)
```

**Arguments**

tiles	A set of tiles representing each group of sequences, as in the format created by the function TileSeqs.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. Determines the target group(s) for which probes will be designed. If "" then all identifiers are selected.
start	Integer specifying the starting position in the alignment where potential target sites begin. Preferably a position that is included in most sequences in the alignment.

end	Integer specifying the ending position in the alignment where potential target sites end. Preferably a position that is included in most sequences in the alignment.
minLength	Integer providing the minimum length of probes to consider in the design.
maxLength	Integer providing the maximum length of probes to consider in the design, which must be less than or equal to the maxLength of tiles.
maxPermutations	Integer providing the maximum number of probe permutations required to reach the desired coverage of a target site.
minCoverage	Numeric giving the minimum fraction of the target group's sequences that must be covered by the designed probe(s).
minGroupCoverage	Numeric giving the minimum fraction of the target group that must have sequence information (not terminal gaps) in the target site's region.
hybTemp	Numeric specifying the hybridization temperature, typically 46 degrees Celsius.
P	Numeric giving the molar concentration of probes during hybridization.
Na	Numeric giving the molar sodium concentration in the hybridization buffer. Values may range between 0.01M and 1M. Note that salt correction from 1 molar is not available for the thermodynamic rules of RNA/RNA interactions.
FA	Numeric concentration (as percent v/v) of the denaturant formamide in the hybridization buffer.
minEfficiency	Numeric giving the minimum equilibrium hybridization efficiency desired for designed probe(s) at the defined experimental conditions.
worstScore	Numeric specifying the score cutoff to remove target sites from consideration. For example, a worstScore of -5 will remove all probes scoring below -5, although this may eventually result in no probes meeting the design criteria.
numProbeSets	Integer giving the optimal number of dual probe sets to design. If set to zero then all potential single probes are returned, and the probe sets minimizing potential false cross-hybridizations are not chosen.
batchSize	Integer specifying the number of probes to simulate hybridization per batch that is passed to CalculateEfficiencyFISH.
target	The target molecule used in the generation of tiles. Either "SSU" for the small-subunit rRNA, "LSU" for the large-subunit rRNA, or "Other". Used to determine the domain for dG3 calculations, which is plus or minus 200 nucleotides of the target site if "Other".
verbose	Logical indicating whether to display progress.

### Details

Probes are designed to maximize sensitivity and specificity to the target group(s) (*identifier(s)*). If *numProbeSets* > 0 then that many pairs of probes with minimal cross-hybridization overlap are returned, enabling increased specificity with a dual-color approach.

Probes are designed from a set of tiles to target each *identifier* while minimizing affinity for all other tiled groups. Arguments provide constraints that ensure the designed probes meet the specified

criteria as well as being optimized for the particular experimental conditions. A search is conducted through all tiles in the same alignment position to estimate the chance of cross-hybridization with a non-target group.

Two models are used in design, both of which were experimentally calibrated using denaturation profiles from 5 organisms belonging to all three domains of life. Probe lengths are chosen to meet the `minEfficiency` using a fast model of probe-target hybridization. Candidate probes are then confirmed using a slower model that also takes into account probe-folding and target-folding. Finally, probes are scored for their inability to cross-hybridize with non-target groups by using the fast model and taking into account any mismatches.

### Value

A different `data.frame` will be returned depending on number of primer sets requested. If no probe sets are required then columns contain the designed probes for every possible position scored by their potential to cross-hybridize with other identified groups. If one or more probe sets are requested then columns contain information for the optimal set of probes (probe one and probe two) that could be used in combination to give the fewest potential cross-hybridizations.

### Note

The program `OligoArrayAux` (<http://mfold.rna.albany.edu/?q=DINAMelt/OligoArrayAux>) must be installed in a location accessible by the system. For example, the following code should print the installed `OligoArrayAux` version when executed from the R console:

```
system("hybrid-min -V")
```

To install `OligoArrayAux` from the downloaded source folder on Unix-like platforms, open the shell (or Terminal on Mac OS) and type:

```
cd oligoarrayaux # change directory to the correct folder name
./configure
make
sudo make install
```

### Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

### References

Coming Soon!

### See Also

[CalculateEfficiencyFISH](#), [TileSeqs](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
# not run (must have OligoArrayAux installed first):
#tiles <- TileSeqs(db, identifier=c("Acinetobacter", "Pseudomonas"))
#probes <- DesignProbes(tiles, identifier="Acinetobacter", start=280, end=420)
```

---

DistanceMatrix                      *Calculate the Distances Between Sequences*

---

**Description**

Calculates a distance matrix for an XStringSet. Each element of the distance matrix corresponds to the dissimilarity between two sequences in the XStringSet.

**Usage**

```
DistanceMatrix(myXStringSet,
               includeTerminalGaps = FALSE,
               penalizeGapLetterMatches = TRUE,
               penalizeGapGapMatches = FALSE,
               removeDuplicates = FALSE,
               correction = "none",
               processors = NULL,
               verbose = TRUE)
```

**Arguments**

myXStringSet	An XStringSet object of aligned sequences (DNAStrngSet, RNAStrngSet, or AAStrngSet).
includeTerminalGaps	Logical specifying whether or not to include terminal gaps ("- characters on each end of the sequence) into the calculation of distance.
penalizeGapLetterMatches	Logical specifying whether or not to consider gap-to-letter matches as mismatches.
penalizeGapGapMatches	Logical specifying whether or not to consider gap-to-gap matches as mismatches.
removeDuplicates	Logical specifying whether to remove any identical sequences from the input sequences before calculating distance. If FALSE (the default) then the distance matrix is calculated with the entire XStringSet provided as input.
correction	The substitution model used for distance correction. This should be (an unambiguous abbreviation of) one of "none" or "Jukes-Cantor".
processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.

## Details

The uncorrected distance matrix represents the percent distance between each of the sequences in the XStringSet. Ambiguity can be represented using the characters of the IUPAC\_CODE\_MAP for DNAStrngSet and RNAStrngSet inputs, or using the AMINO\_ACID\_CODE for an AAStrngSet input. For example, the distance between an 'N' and any other nucleotide base is zero. The letters B (N or D), Z (Q or E), and X (any letter) are degenerate in the AMINO\_ACID\_CODE.

If includeTerminalGaps = FALSE then terminal gaps are not included in sequence length. This can be faster since only the positions common to each pair of sequences are compared. Similarly, if removeDuplicates = TRUE then the distance matrix will only represent unique sequences in the XStringSet. This is can be faster because less sequences need to be compared. For example, if two sequences in the set are exact duplicates then one is removed and the distance is calculated on the remaining set. Note that the distance matrix can still contain values of 100% after removing duplicates because only exact duplicates are removed without taking into account ambiguous matches or the treatment of gaps.

The elements of the distance matrix can be referenced by dimnames corresponding to the names of the XStringSet. Additionally, an attribute named "correction" specifying the method of correction used can be accessed using the function attr.

## Value

A symmetric matrix where each element is the distance between the sequences referenced by the respective row and column. The dimnames of the matrix correspond to the names of the XStringSet. Sequences with no overlapping positions in the alignment are given a value of NA.

## Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

## See Also

[IdClusters](#)

## Examples

```
# defaults compare intersection of internal ranges:
dna <- DNAStrngSet(c("ANGCT-", "-ACCT-"))
d <- DistanceMatrix(dna)
# d[1,2] is still 1 base in 4 = 0.25

# compare union of internal ranges:
dna <- DNAStrngSet(c("ANGCT-", "-ACCT-"))
d <- DistanceMatrix(dna, includeTerminalGaps=TRUE)
# d[1,2] is now 2 bases in 5 = 0.40

# compare the entire sequence ranges:
dna <- DNAStrngSet(c("ANGCT-", "-ACCT-"))
d <- DistanceMatrix(dna, includeTerminalGaps=TRUE,
                    penalizeGapGapMatches=TRUE)
# d[1,2] is now 3 bases in 6 = 0.50
```

---

FindChimeras

*Find Chimeras In A Sequence Database*


---

### Description

Finds chimeras present in a database of sequences. Makes use of a reference database of (presumed to be) good quality sequences.

### Usage

```
FindChimeras(dbFile,
             tblName = "DNA",
             identifier = "",
             dbFileReference,
             batchSize = 100,
             minNumFragments = 20000,
             tb.width = 5,
             multiplier = 20,
             minLength = 70,
             minCoverage = 0.6,
             overlap = 100,
             minSuspectFragments = 6,
             showPercentCoverage = FALSE,
             add2tbl = FALSE,
             maxGroupSize = -1,
             minGroupSize = 100,
             verbose = TRUE)
```

### Arguments

dbFile	A SQLite connection object or a character string specifying the path to the database file to be checked for chimeric sequences.
tblName	Character string specifying the table in which to check for chimeras.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" then all identifiers are selected.
dbFileReference	A SQLite connection object or a character string specifying the path to the reference database file of (presumed to be) good quality sequences. A 16S reference database is available from <a href="http://DECIPHER.cee.wisc.edu">DECIPHER.cee.wisc.edu</a> .
batchSize	Number sequences to tile with fragments at a time.
minNumFragments	Number of suspect fragments to accumulate before searching through other groups.
tb.width	A single integer [1..14] giving the number of nucleotides at the start of each fragment that are part of the trusted band.

multiplier	A single integer specifying the multiple of fragments found out-of-group greater than fragments found in-group in order to consider a sequence a chimera.
minLength	Minimum length of a chimeric region in order to be considered as a chimera.
minCoverage	Minimum fraction of coverage necessary in a chimeric region.
overlap	Number of nucleotides at the end of the sequence that the chimeric region must overlap in order to be considered a chimera.
minSuspectFragments	Minimum number of suspect fragments belonging to another group required to consider a sequence a chimera.
showPercentCoverage	Logical indicating whether to list the percent coverage of suspect fragments in each chimeric region in the output.
add2tbl	Logical or a character string specifying the table name in which to add the result.
maxGroupSize	Maximum number of sequences searched in a group. A value of less than 0 means the search is unlimited.
minGroupSize	The minimum number of sequences in a group to be considered as part of the search for chimeras. May need to be set to a small value for reference database with mostly small groups.
verbose	Logical indicating whether to display progress.

## Details

The algorithm works by finding suspect fragments that are uncommon in the group where the sequence belongs, but very common in another group where the sequence does not belong. Each sequence in the `dbFile` is tiled into short sequence segments called fragments. If the fragments are infrequent in their respective group in the `dbFileReference` then they are considered suspect. If enough suspect fragments from a sequence meet the specified constraints then the sequence is flagged as a chimera.

The default parameters are optimized for full-length 16S sequences (> 1,000 nucleotides). Shorter 16S sequences require two parameters that are different than the defaults: `minLength` = 40, and `minSuspectFragments` = 2.

Groups are determined by the identifier present in each database. For this reason, the groups in the `dbFile` should exist in the groups of the `dbFileReference`. The reference database is assumed to contain many sequences of only good quality.

If a reference database is not present then it is feasible to create a reference database by using the input database as the reference database. Removing chimeras from the reference database and then iteratively repeating the process can result in a clean reference database.

For non-16S sequences it may be necessary to optimize the parameters for the particular sequences. The simplest way to perform an optimization is to experiment with different input parameters on artificial chimeras such as those created using `CreateChimeras`. Adjusting input parameters until the maximum number of artificial chimeras are identified is the easiest way to determine new defaults.

**Value**

A data.frame containing only the sequences that meet the specifications for being chimeric. The chimera column contains information on the chimeric region and to which group it belongs. The row.names of the data.frame correspond to those of the sequences in dbFile.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**References**

ES Wright et al. (2012) "DECIPHER: A Search-Based Approach to Chimera Identification for 16S rRNA Sequences." Applied and Environmental Microbiology, doi:10.1128/AEM.06516-11.

**See Also**

[CreateChimeras](#), [Add2DB](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
# It is necessary to set dbFileReference to the file path of the
# 16S reference database available from DECIPHER.cce.wisc.edu
chimeras <- FindChimeras(db, dbFileReference=db)
```

---

FormGroups

*Forms Groups By Rank*

---

**Description**

Agglomerates sequences into groups in a certain size range based on taxonomic rank.

**Usage**

```
FormGroups(dbFile,
           tblName = "DNA",
           goalSize = 1000,
           minGroupSize = 500,
           maxGroupSize = 10000,
           add2tbl = FALSE,
           verbose = TRUE)
```

**Arguments**

dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table where the rank information is located.
goalSize	Number of sequences required in each group to stop adding more sequences.
minGroupSize	Minimum number of sequences in each group required to stop trying to recombine with a larger group.
maxGroupSize	Maximum number of sequences in each group allowed to continue agglomeration.
add2tbl	Logical or a character string specifying the table name in which to add the result.
verbose	Logical indicating whether to print database queries and other information.

**Details**

FormGroups uses the “rank” field in the dbFile table to group sequences with similar taxonomic rank. Requires that rank information be present in the tblName, such as that created when importing sequences from a GenBank file. The rank information must not contain repeated names belonging to different lineages.

Beginning with the least common ranks, the algorithm agglomerates groups with similar ranks until the goalSize is reached. If the group size is below minGroupSize then further agglomeration is attempted with a larger group. If additional agglomeration results in a group larger than maxGroupSize then the agglomeration is undone so that the group is smaller.

**Value**

Returns a data.frame of rank and id for each group. If add2tbl is not FALSE then the tblName is updated with the group as the identifier.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[IdentifyByRank](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
g <- FormGroups(db, goalSize=10, minGroupSize=5, maxGroupSize=20)
```

**Description**

Groups the sequences represented by a distance matrix into clusters of similarity.

**Usage**

```
IdClusters(myDistMatrix = NULL,
          method = "UPGMA",
          cutoff = -Inf,
          showPlot = FALSE,
          asDendrogram = FALSE,
          myXStringSet = NULL,
          model = MODELS,
          add2tbl = FALSE,
          dbFile = NULL,
          processors = NULL,
          verbose = TRUE)
```

**Arguments**

myDistMatrix	A symmetric $N \times N$ distance matrix with the values of dissimilarity between $N$ sequences, or NULL if method is "inexact".
method	An agglomeration method to be used. This should be (an unambiguous abbreviation of) one of "complete", "single", "UPGMA", "WPGMA", "NJ", "ML", or "inexact". (See details section below.)
cutoff	A vector with the maximum edge length separating the sequences in the same cluster. Multiple cutoffs may be provided in ascending order. If asDendrogram=TRUE or showPlot=TRUE then only one cutoff may be specified.
showPlot	Logical specifying whether or not to plot the resulting dendrogram. Not applicable if method=inexact.
asDendrogram	Logical. If TRUE then the object returned is of class dendrogram. Not applicable if method=inexact.
myXStringSet	If method is "ML", the DNAStrngSet used in the creation of myDistMatrix. If method is "inexact", the DNAStrngSet, RNAStrngSet, or AAStrngSet to cluster. Not applicable for other methods.
model	One or more of the available MODELS of DNA evolution. Only applicable if method is "ML".
add2tbl	Logical or a character string specifying the table name in which to add the result.
dbFile	A connection to a SQLite database or character string giving the path to the database file. Only necessary if add2tbl is not FALSE.
processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.

## Details

Groups the input sequences into clusters using a set of dissimilarities representing the distance between  $N$  sequences. Initially a phylogenetic tree is formed using the specified method. Then each leaf (sequence) of the tree is assigned to a cluster based on its edge lengths to the other sequences. These clustering methods are described as follows:

**Ultrametric methods:** The method `complete` assigns clusters using complete-linkage so that sequences in the same cluster are no more than `cutoff` percent apart. The method `single` assigns clusters using single-linkage so that sequences in the same cluster are within `cutoff` of at least one other sequence in the same cluster. `UPGMA` (the default) or `WPGMA` assigns clusters using average-linkage which is a compromise between the sensitivity of complete-linkage clustering to outliers and the tendency of single-linkage clustering to connect distant relatives that do not appear to be closely related. `UPGMA` produces an unweighted tree, where each element contributes equally to the average edge lengths, whereas `WPGMA` produces a weighted result.

**Additive methods:** `NJ` uses the Neighbor-Joining method proposed by Saitou and Nei that does not assume lineages evolve at the same rate (the molecular clock hypothesis). The `NJ` method is typically the most phylogenetically accurate of the above distance-based methods. `ML` creates a neighbor-joining tree and then iteratively maximizes the likelihood of the tree given the aligned sequences (`myXStringSet`). This is accomplished through a combination of optimizing edge lengths with Brent's method and improving tree topology with nearest-neighbor interchanges (NNIs). When `method="ML"`, one or more `MODELS` of DNA evolution must be specified. Model parameters are iteratively optimized to maximize likelihood, except base frequencies which are empirically determined. If multiple models are given, the best model is automatically chosen based on BIC calculated from the likelihood and the sample size (defined as the number of variable sites in the DNA sequence).

**Sequence-only method:** `inexact` uses a greedy incremental algorithm to directly assign sequences to clusters without a distance matrix. First the sequences are ordered by length and the longest sequence becomes the first cluster seed. If the second sequence is less than `cutoff` percent distance then it is added to the cluster, otherwise it becomes a new cluster representative. The remaining sequences are matched to cluster representatives using an ordered k-mer strategy, and then compared to the top hits with pairwise alignment. This approach, finding the closest cluster representatives followed by pairwise alignment to obtain the percent identity, is repeated until all sequences belong to a cluster. If multiple cutoffs are specified then clustering is continued within each cluster as the cutoff decreases. In this way individual clusters at lower values of `cutoff` are completely contained within their umbrella clusters at higher values of `cutoff`. This process results in clusters with members generally separated by less than `cutoff` distance.

If `add2tbl=TRUE` then the resulting `data.frame` is added/updated into column(s) of the default table "DNA" in `dbFile`. If `add2tbl` is a character string then the result is added to the specified table name in `dbFile`. The added/updated column names are printed if `verbose=TRUE`.

## Value

If `asDendrogram=FALSE` (the default), returns a `data.frame` with a column for each cutoff specified. The `row.names` of the `data.frame` correspond to the `dimnames` of `myDistMatrix`. Each one of  $N$  sequences is assigned to one of  $M$  clusters. If `asDendrogram=TRUE`, returns an object of class `dendrogram` that can be used for further manipulation and plotting. Leaves of the dendrogram are randomly colored by cluster number.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**References**

- Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, **17(6)**, 368-376.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4(4)**, 406-425.
- Ghods, M., Liu, B., & Pop, M. (2011) DNACLUST. *BMC Bioinformatics*, **12(1)**, 271. doi:10.1186/1471-2105-12-271.

**See Also**

[DistanceMatrix](#), [Add2DB](#), [MODELS](#)

**Examples**

```
# using the matrix from the original paper by Saitou and Nei
m <- matrix(0,8,8)
m[2:8,1] <- c(7, 8, 11, 13, 16, 13, 17)
m[3:8,2] <- c(5, 8, 10, 13, 10, 14)
m[4:8,3] <- c(5, 7, 10, 7, 11)
m[5:8,4] <- c(8, 11, 8, 12)
m[6:8,5] <- c(5, 6, 10)
m[7:8,6] <- c(9, 13)
m[8,7] <- c(8)

# returns an object of class "dendrogram"
myClusters <- IdClusters(m, cutoff=10, method="NJ", showPlot=TRUE, asDendrogram=TRUE)

# example of specifying a cutoff
# returns a data frame
IdClusters(m, cutoff=c(2,6,10,20))
```

**Description**

Forms a consensus sequence representing the sequences in each group.

**Usage**

```
IdConsensus(dbFile,  
            tblName = "DNA",  
            identifier = "",  
            type = "DNAStringSet",  
            colName = "id",  
            add2tbl = FALSE,  
            verbose = TRUE,  
            ...)
```

**Arguments**

dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table in which to form consensus.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" then all identifiers are selected.
type	The type of XStringSet (sequences) to use in forming consensus. This should be (an unambiguous abbreviation of) one of "DNAStringSet", "RNAStringSet", "AAStringSet", or "BStringSet".
colName	Column containing the group name of each sequence.
add2tbl	Logical or a character string specifying the table name in which to add the result.
verbose	Logical indicating whether to display progress.
...	Additional arguments to be passed directly to <a href="#">ConsensusSequence</a> for an AAStringSet, DNAStringSet, or RNAStringSet, or to consensusString for a BStringSet.

**Details**

Creates a consensus sequence for each of the distinct groups defined in colName. The resulting XStringSet contains as many consensus sequences as there are groups in colName. For example, it is possible to create a set of consensus sequences with one consensus sequence for each "id", or the "cluster" output of IdClusters.

**Value**

An XStringSet object containing the consensus sequence for each group. The names of the XStringSet contain the number of sequences and name of each group.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[Seqs2DB](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
con <- IdConsensus(db, colName="id", noConsensusChar="N")
BrowseSequences(con)
```

---

IdentifyByRank	<i>Identify By Taxonomic Rank</i>
----------------	-----------------------------------

---

**Description**

Identifies sequences by a specific level of their taxonomic rank.

**Usage**

```
IdentifyByRank(dbFile,
               tblName = "DNA",
               level = 3,
               add2tbl = FALSE,
               verbose = TRUE)
```

**Arguments**

dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table where the rank information is located.
level	Level of the taxonomic rank. (See details section below.)
add2tbl	Logical or a character string specifying the table name in which to add the result.
verbose	Logical indicating whether to print database queries and other information.

**Details**

Simply identifies a sequence by a specific level of its taxonomic rank. Requires that rank information be present in the tblName, such as that created when importing sequences from a GenBank file.

The input parameter level should be a non-zero integer giving the “level” of the taxonomic rank to choose as the identifier. Negative levels are interpreted as that many levels from the final level.

If the specified level of rank does not exist then the closest rank is chosen. This makes it possible to determine the lowest level classification (e.g., genus) by specifying level = Inf.

**Value**

A data.frame with the rank and corresponding identifier as "id".

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**[FormGroups](#)**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
ids <- IdentifyByRank(db)
```

---

IdLengths	<i>Determine the Number of Bases, Nonbases, and Width of Each Sequence</i>
-----------	--

---

**Description**

Counts the number of bases (A, C, G, T) and ambiguities/degeneracies in each sequence.

**Usage**

```
IdLengths(dbFile,
          tblName = "DNA",
          identifier = "",
          type = "DNAStrngSet",
          add2tbl = FALSE,
          batchSize = 10000,
          verbose = TRUE)
```

**Arguments**

dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table where the sequences are located.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" then all identifiers are selected.
type	The type of XStringSet being processed. This should be (an unambiguous abbreviation of) one of "DNAStrngSet" or "RNAStrngSet".
add2tbl	Logical or a character string specifying the table name in which to add the result.
batchSize	Integer specifying the number of sequences to process at a time.
verbose	Logical indicating whether to display progress.

**Value**

A data.frame with the number of bases, nonbases, and width of each sequence. The width is defined as the sum of bases and nonbases in each sequence. The row.names of the data.frame correspond to the "row\_names" in the tblName of the dbFile.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[Add2DB](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
l <- IdLengths(db)
```

---

MaskAlignment

*Masks Highly Variable Regions of An Alignment*

---

**Description**

Automatically masks poorly aligned regions of an alignment based on sequence conservation and gap frequency.

**Usage**

```
MaskAlignment(myXStringSet,
              windowSize = 5,
              threshold = 1,
              maxFractionGaps = 0.2,
              showPlot = FALSE)
```

**Arguments**

myXStringSet	An AStringSet, DNASTringSet, or RNASTringSet object of aligned sequences.
windowSize	Integer value specifying the size of the center-point moving average to use in determining variable regions.
threshold	Numeric giving the average entropy in bits from 0 to 2 below which a region is masked.
maxFractionGaps	Numeric specifying the maximum fraction of gaps in an alignment column to be masked.
showPlot	Logical specifying whether or not to show a plot of the positions that were kept or masked.

## Details

Poorly aligned regions of a multiple sequence alignment may lead to incorrect results in downstream analyses, and require extra processing time. One method to mitigate their effects is to mask columns of the alignment that may be poorly aligned, such as highly-variable regions or regions with many insertions and deletions (gaps).

Highly variable regions are detected by their signature of having low information content. A moving average of windowSize nucleotides to the left and right of the center-point is applied to smooth noise in the information content signal along the sequence. Regions dropping below threshold bits or more than maxFractionGaps are masked in the returned alignment.

## Value

An XMultipleAlignment object of the input type with masked columns where the input criteria are met.

## Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

## See Also

[AlignSeqs](#), [IdClusters](#)

## Examples

```
fas <- system.file("extdata", "Streptomyces_ITS_aligned.fas", package="DECIPHER")
dna <- readDNASTringSet(fas)
masked_dna <- MaskAlignment(dna, showPlot=TRUE)

# display only unmasked nucleotides for use in downstream analyses
not_masked <- as(masked_dna, "DNASTringSet")
BrowseSequences(not_masked)

# display only masked nucleotides that are covered by the mask
masked <- masked_dna
colmask(masked, append="replace", invert=TRUE) <- colmask(masked)
masked <- as(masked, "DNASTringSet")
BrowseSequences(masked)

# display the complete DNA sequence set including the mask
masks <- lapply(width(colmask(masked_dna)), rep, x="+")
masks <- unlist(lapply(masks, paste, collapse=""))
masked_dna <- replaceAt(dna, at=IRanges(colmask(masked_dna)), value=masks)
BrowseSequences(masked_dna)
```

**Description**

The MODELS character vector contains the models of DNA evolution that can be used by IdClusters.

**Usage**

MODELS

**Details**

Six models of DNA evolution are available, with or without the discrete Gamma rates distribution. These are described in order of increasing number of parameters as follows:

**JC69 (Jukes and Cantor, 1969)** The simplest substitution model that assumes equal base frequencies (1/4) and equal mutation rates.

**K80 (Kimura, 1980)** Assumes equal base frequencies, but distinguishes between the rate of transitions and transversions.

**T92 (Tamura, 1992)** In addition to distinguishing between transitions and transversions, a parameter is added to represent G+C content bias.

**F81 (Felsenstein, 1981)** Assumes equal mutation rates, but allows all bases to have different frequencies.

**HKY85 (Hasegawa, Kishino and Yano, 1985)** Distinguishes transitions from transversions and allows bases to have different frequencies.

**TN93 (Tamura and Nei, 1993)** Allows for unequal base frequencies and distinguishes between transversions and the two possible types of transitions (i.e., A <-> G & C <-> T).

**+G (Yang, 1993)** Specifying a model+G4 adds a single parameter to any of the above models to relax the assumption of equal rates among sites in the DNA sequence. The single parameter specifies the shape of the Gamma Distribution. The continuous distribution is represented with 2-10 discrete rates and their respective probabilities as determined by the Laguerre Quadrature method (Felsenstein, 2001). For example, specifying a model+G8 would represent the continuous Gamma Distribution with eight rates and their associated probabilities.

**References**

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, **17(6)**, 368-376.

Felsenstein, J. (2001). Taking Variation of Evolutionary Rates Between Sites into Account in Inferring Phylogenies. *Journal of molecular evolution*, **53(4-5)**, 447-455.

Hasegawa, M., Kishino H., Yano T. (1985). Dating of human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22(2)**, 160-174.

Jukes, T. and Cantor C. (1969). *Evolution of Protein Molecules*. New York: Academic Press. pp. 21-132.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**(2), 111-120.

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C content biases. *Molecular Biology and Evolution*, **9**(4), 678-687.

Tamura, K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**(3), 512-526.

Yang, Z. (1993). Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution*, **10**(6), 1396-1401.

### See Also

[IdClusters](#)

### Examples

MODELS

---

NNLS	<i>Sequential Coordinate-wise Algorithm for the Non-negative Least Squares Problem</i>
------	--

---

### Description

Consider the linear system  $\mathbf{A}x = b$  where  $\mathbf{A} \in R^{m \times n}$ ,  $x \in R^n$ , and  $b \in R^m$ . The technique of least squares proposes to compute  $x$  so that the sum of squared residuals is minimized. NNLS solves the least squares problem  $\min \|\mathbf{A}x - b\|^2$  subject to the constraint  $x \geq 0$ . This implementation of the Sequential Coordinate-wise Algorithm uses a sparse input matrix  $\mathbf{A}$ , which makes it efficient for large sparse problems.

### Usage

```
NNLS(A,
      b,
      precision = sqrt(.Machine$double.eps),
      processors = NULL,
      verbose = TRUE)
```

### Arguments

A	List representing the sparse matrix with integer components i and j, numeric component x. The fourth component, dimnames, is a list of two components that contains the names for every row (component 1) and column (component 2).
b	Numeric matrix for the set of observed values. (See details section below.)
precision	The desired accuracy.

processors	The number of processors to use, or NULL (the default) for all available processors.
verbose	Logical indicating whether to display progress.

### Details

The input  $b$  can be either a matrix or a vector of numerics. If it is a matrix then it is assumed that each column contains a set of observations, and the output  $x$  will have the same number of columns. This allows multiple NNLS problems using the same  $A$  matrix to be solved simultaneously, and greatly accelerates computation relative to solving each sequentially.

### Value

A list of two components:

$x$	The matrix of non-negative values that best explains the observed values given by $b$ .
res	A matrix of residuals given by $Ax - b$ .

### References

Franc, V., et al. (2005). Sequential coordinate-wise algorithm for the non-negative least squares problem. *Computer Analysis of Images and Patterns*, 407-414.

### See Also

[Array2Matrix](#), [DesignArray](#)

### Examples

```
# unconstrained least squares:
A <- matrix(c(1, -3, 2, -3, 10, -5, 2, -5, 6), ncol=3)
b <- matrix(c(27, -78, 64), ncol=1)
x <- solve(crossprod(A), crossprod(A, b))

# Non-negative least squares:
w <- which(A > 0, arr.ind=TRUE)
A <- list(i=w[,"row"], j=w[,"col"], x=A[w,
      dimnames=list(1:dim(A)[1], 1:dim(A)[2]))
x_nonneg <- NNLS(A, b)

# compare the unconstrained and constrained solutions:
cbind(x, x_nonneg$x)

# the input value "b" can also be a matrix:
b2 <- matrix(b, nrow=length(b), ncol=2) # repeat b in two columns
x_nonneg <- NNLS(A, b2) # solution is repeated in two output columns
```

---

RESTRICTION\_ENZYMES     *Common Restriction Sites Named By Restriction Enzyme*

---

**Description**

A character vector of common restriction sites named by the restriction enzyme(s) that cut at each site.

**Usage**

```
data(RESTRICTION_ENZYMES)
```

**Format**

The format is: Named chr [1:233] "CGGCCG" "ATCGAT" "CACCTGC" ... - attr(\*, "names")= chr [1:233] "AaaI/BseX3I/BstZI/EagI/"| \_\_truncated\_\_ "AagI/BanII/BavCI/BbvAII/"| \_\_truncated\_\_ "AarI" "AasI/DrdI/DseDI" ...

**Source**

List of restriction enzymes available from: <http://www.genscript.com/>.

**Examples**

```
data(RESTRICTION_ENZYMES)
```

---

SearchDB     *Obtain Specific Sequences from A Database*

---

**Description**

Returns the set of sequences meeting the search criteria.

**Usage**

```
SearchDB(dbFile,
         tblName = "DNA",
         identifier = "",
         type = "DNAStrngSet",
         limit = -1,
         replaceChar = "-",
         nameBy = "row_names",
         orderBy = "row_names",
         countOnly = FALSE,
         removeGaps = "none",
         clause = "",
         verbose = TRUE)
```

**Arguments**

dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table where the sequences are located.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" (the default) then all identifiers are selected.
type	The type of XStringSet (sequences) to return. This should be (an unambiguous abbreviation of) one of "DNAStrngSet", "RNAStrngSet", "AAStrngSet", "BStringSet", "QualityScaledDNAStrngSet", "QualityScaledRNAStrngSet", "QualityScaledAAStrngSet", or "QualityScaledBStringSet".
limit	Number of results to display. The default (-1) does not limit the number of results.
replaceChar	Optional character used to replace any characters of the sequence that are not present in the XStringSet's alphabet. Not applicable if type=="BStringSet". (See details section below.)
nameBy	Character string giving the column name for naming the XStringSet.
orderBy	Character string giving the column name for sorting the results. Defaults to the order of entries in the database. Optionally can be followed by " ASC" or " DESC" to specify ascending (the default) or descending order.
countOnly	Logical specifying whether to return only the number of sequences.
removeGaps	Determines how gaps are removed in the sequences. This should be (an unambiguous abbreviation of) one of "none", "all" or "common".
clause	An optional character string to append to the query as a clause.
verbose	Logical indicating whether to display queries as they are sent to the database.

**Details**

If type is "DNAStrngSet" then all U's are converted to T's before creating the DNAStrngSet, and vice-versa if type is "RNAStrngSet". All remaining characters not in the XStringSet's alphabet are converted to replaceChar. Quality information is interpreted as PredQuality scores.

**Value**

An XStringSet or QualityScaledXStringSet with the sequences that meet the specified criteria. The names of the object correspond to the value in the nameBy column of the database.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[Seqs2DB](#), [DB2Seqs](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
dna <- SearchDB(db)
```

Seqs2DB

*Add Sequences from Text File to Database***Description**

Adds sequences to a database.

**Usage**

```
Seqs2DB(seqs,
        type,
        dbFile,
        identifier,
        tblName = "DNA",
        chunkSize = 1e5,
        replaceTbl = FALSE,
        verbose = TRUE)
```

**Arguments**

seqs	A connection object or a character string specifying the file path to the file containing the sequences, an XStringSet object if type is XStringSet, or a QualityScaledXStringSet object if type is QualityScaledXStringSet.
type	The type of the sequences (seqs) being imported. This should be (an unambiguous abbreviation of) one of "FASTA", "FASTQ", "GenBank", "XStringSet", or "QualityScaledXStringSet".
dbFile	A SQLite connection object or a character string specifying the path to the database file. If the dbFile does not exist then a new database is created at this location.
identifier	Character string specifying the "id" to give the imported sequences in the database.
tblName	Character string specifying the table in which to add the sequences.
chunkSize	Number of lines of the seqs to read at a time.
replaceTbl	Logical. If FALSE (the default) then the sequences are appended to any already existing in the table. If TRUE then any sequences already in the table are overwritten.
verbose	Logical indicating whether to display each query as it is sent to the database.

**Details**

Sequences are imported into the database in chunks of lines specified by chunkSize. The sequences can then be identified by searching the database for the identifier provided. Sequences are added to the database verbatim, so that no sequence information is lost when the sequences are exported from the database.

**Value**

The total number of sequences in the database table is returned after import.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[SearchDB](#), [DB2Seqs](#)

**Examples**

```
gen <- system.file("extdata", "Bacteria_175seqs.gen", package="DECIPHER")
dbConn <- dbConnect(SQLite(), ":memory:")
Seqs2DB(gen, "GenBank", dbConn, "Bacteria")
BrowseDB(dbConn)
dbDisconnect(dbConn)
```

---

TerminalChar

*Determine the Number of Terminal Characters*

---

**Description**

Counts the number of terminal characters for every sequence in an XStringSet. Terminal characters are defined as a specific character repeated at the beginning and end of a sequence.

**Usage**

```
TerminalChar(myXStringSet,
             char = "-")
```

**Arguments**

myXStringSet    An XStringSet object of sequences.  
char            A single character giving the terminal character to count.

**Value**

A matrix containing the results for each sequence in its respective row. The first column contains the number of leading char, the second contains the number of trailing char, and the third contains the total number of characters in between.

**Author(s)**

Erik Wright <DECIPHER@cae.wisc.edu>

**See Also**

[IdLengths](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
dna <- SearchDB(db)
t <- TerminalChar(dna)
```

---

TileSeqs

*Form a Set of Tiles for Each Group of Sequences.*

---

**Description**

Creates a set of tiles that represent each group of sequences in the database for downstream applications.

**Usage**

```
TileSeqs(dbFile,
         tblName = "DNA",
         identifier = "",
         minLength = 26,
         maxLength = 27,
         maxTilePermutations = 10,
         minCoverage = 0.9,
         add2tbl = FALSE,
         verbose = TRUE,
         ...)
```

**Arguments**

dbFile	A SQLite connection object or a character string specifying the path to the database file.
tblName	Character string specifying the table of sequences to use for forming tiles.
identifier	Optional character string used to narrow the search results to those matching a specific identifier. If "" then all identifiers are selected.
minLength	Integer providing the minimum number of nucleotides in each tile. Typically the same or slightly less than maxLength.
maxLength	Integer providing the maximum number of nucleotides in each tile. Tiles are designed primarily for this length, which should ideally be slightly greater than the maximum length of oligos used in downstream functions.
maxTilePermutations	Integer specifying the maximum number of tiles in each target site.

<code>minCoverage</code>	Numeric providing the fraction of coverage that is desired for each target site in the group. For example, a <code>minCoverage</code> of 0.9 request that additional tiles are added until 90% of the group is represented by the tile permutations.
<code>add2tbl</code>	Logical or a character string specifying the table name in which to add the result.
<code>verbose</code>	Logical indicating whether to display progress.
<code>...</code>	Additional arguments to be passed directly to SearchDB.

### Details

This function will create a set of overlapping tiles representing each target site in an alignment of sequences. The most common tile permutations are added until the minimum group coverage is obtained. The `dbFile` is assumed to contain `DNAStrngSet` sequences (any U's are converted to T's).

Target sites with one more more tiles not meeting a set of requirements are marked with `misprime` equals `TRUE`. Requirements are a minimum group coverage, minimum length, and a maximum length. Additionally, tiles are required not to contain more than four runs of a single base or four di-nucleotide repeats.

### Value

A `data.frame` with a row for each tile, and multiple columns of information. The `row_names` column gives the row number. The `start`, `end`, `start_aligned`, and `end_aligned` columns provide positioning of the tile in a consensus sequence formed from the group. The column `misprime` is a logical specifying whether the tile meets the specified constraints. The columns `width` and `id` indicate the tiles length and group of origin, respectively.

The `coverage` field gives the fraction of sequences containing the tile in the group that encompass the tiles start and end positions in the alignment, whereas the `groupCoverage` contains the fraction of all sequences in the group containing a tile at their respective target site. For example, if 100% of sequences have a certain tile permutation in the first alignment position, but only a single sequence includes this position in the alignment then `coverage` would be 100% (1.0), while `groupCoverage` would be 10% (0.1).

The final column, `target_site`, provides the sequence of the tile.

### Note

If `add2tbl` is `TRUE` then the tiles will be added to the database table that currently contains the sequences used for tiling. The added tiles may cause interference when querying a table of sequences. Therefore, it is recommended to add the tiles to their own table, for example, by using `add2tbl="Tiles"`.

### Author(s)

Erik Wright <DECIPHER@cae.wisc.edu>

### See Also

[DesignPrimers](#)

**Examples**

```
db <- system.file("extdata", "Bacteria_175seqs.sqlite", package="DECIPHER")
tiles <- TileSeqs(db, identifier="Pseudomonas")
```

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