

BioTools: Tools based on Biostrings (alignment, classification, database)

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Abstract

There are many stand-alone tools available for Bioinformatics. This package aims at using R and the Biostrings package as the common interface for several important tools for multiple sequence alignment (clustalw, kalign), classification (RDP), sequence retrieval (BLAST) as well as database driven sequence management for 16S rRNA.

Keywords: bioinformatics, Bioconductor, biostrings, sequence alignment, sequence classification, sequence management.

1. Introduction

There are many tools available for sequence alignment and classification. Some tools are: BALibase (Smith and Waterman 1981), BLAST (Altschul, Gish, Miller, Myers, and Lipman 1990), T-Coffee (Notredame, Higgins, and Heringa 2000), MAFFT (Katoh, Misawa, Kuma, and Miyata 2002), MUSCLE (Edgar 2004b,a), Kalign (Lassmann and Sonnhammer 2006) and ClustalW2 and ClustalX2 (Larkin, Blackshields, Brown, Chenna, McGettigan, McWilliam, Valentin, Wallace, Wilm, Lopez, Thompson, Gibson, and Higgins 2007). Typically, these tools have a command-line interface and the input and output data is stored in files using various formats. Also the parameters supplied to the command-line interface are different. All this makes using and comparing several approaches time consuming and error prone. The R-based Bioconductor project (?) provides important infrastructure to handle and manipulate bioinformatics data. The **Biostrings** package in particular provides infrastructure for DNA, RNA and protein sequences as well as (multiple) alignments. Also algorithms for sequence alignment are included. However, for multiple sequence alignment and using BLAST the user needs to export the data into a file and then run the needed tool manually and re-import the results. Also, **Biostrings** stores sets of sequences in memory and does not directly support storing and querying classification information.

In **BioTools** we provide a simple interface to a growing set of popular tools. The tools are called directly from within R and no manual data export or import is needed. Currently we interface *clustalw*, *kalign*, *RDP* and *clustalw*. **BioTools** also provides database backed sequence management. Where large amounts of sequences and classification information can be stored and used for selective sequence retrieval.

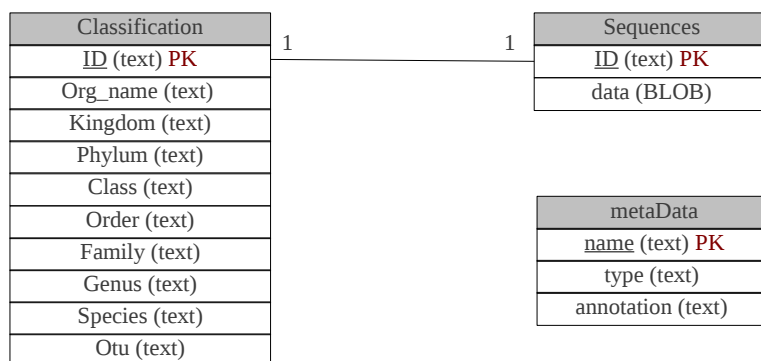


Figure 1: Entity Relationship diagram of GenDB

2. GenDB: Sequence storage and management

BioTools provides a databases (GenDB) which can be used for efficient storage and retrieval of genetic sequences. By default the light-weight SQLite database is used, but any other compatible database such as MySQL or Oracle can also be used. Figure 1 shows the basic table layout of a GenDB instance with a table containing classification information, a table containing the sequence information and a meta data table. Each sequence we will have an entry in the classification table and an corresponding entry in the sequence table. The tables are connected by a unique sequence ID as the primary key.

GenDB is easy to use. First, we load the library into the R environment.

```
R> library(BioTools)
```

To start we need to create an empty GenDB to store and organize sequences.

```
R> db<-createGenDB("example.sqlite")
R> db
```

```
Object of class GenDB with 0 sequences
DB File: example.sqlite
Tables: sequences
```

The above command creates an empty database with a table structure similar to Figure 1 and stores it in the file example.sqlite. If a GenDB already exists, then it can be opened using `openGenDB()`.

The next step is to import sequences into the database by reading FASTA files. This is accomplished by function `addSequences()`. This function automatically extracts the classification information from the FASTA file's description lines. The default is to expect classification in the format used by the Greengenes project, however other meta data readers can be implemented (see manual page for `addSequences`).

The command below uses a FASTA file provided by the package, hence we use `system.file()` instead of just a string with the file name.

```
R> addSequences(db,
+   system.file("examples/Firmicutes.fasta", package="BioTools"))
```

```
Read 100 entries. Added 100 entries.
```

After inserting the sequences, various querying and limiting functions can be used to check the data and obtain a subset of the sequences. To get a count of the number of sequences in the database, the function `nSequences()` can be used.

```
R> nSequences(db)
```

```
[1] 100
```

The function `getSequences()` returns the sequences as a vector. In the following example we get all sequences in the database and then show the first 50 bases of the first sequence.

```
R> s <- getSequences(db)
R> s
```

```
A DNASTringSet instance of length 100
```

	width	seq	names
[1]	1521	TTTGATCCTGGCTCAGG...CGGCTGGATCACCTCCT	1250
[2]	1392	ACGGGTGAGTAACGCGT...TTGGGTGAAGTCGTAA	13651
[3]	1384	TAGTGGCGGACGGGTGA...TCGAATTTGGGTCAAGT	13652
[4]	1672	GGCGTGCCTAACACATG...TGTAACACGACTTCAT	13654
[5]	1386	ATCTCACCTCTCAATAG...CGAAGGTGGGGTTGGTG	13655
[6]	1438	GCGGACGGGTGAGTAAC...GCTGGATCACCTCCTTA	13657
[7]	1392	ACGGGTGAGTAACGCGT...TTGGGTGAAGTCGTAA	13658
[8]	1526	AGAGTTTGATCCTGGCT...GCTGGATCACCTCCTTA	13659
[9]	1440	ATCTCACCTCTCAATAG...GCTGGATCACCTCCTTA	13661
...	
[92]	1516	GGCTCAGGACGAACGCT...GTAGCCGTTTCGAGAACG	13852
[93]	1506	CGAACGCTGGCGGCGTG...GTAGCCGNTTCGAGAACG	13853
[94]	1505	ATCCTGGCTCAGGACGA...AGTCGTAACAAGGTAGC	13855
[95]	1447	ATGCAAGTCGAACGGGG...GGGGCCGATGATTGGGG	13856
[96]	1446	ATGCAAGTCGAACGGGG...GGGGCCGATGATTGGGG	13857
[97]	1511	ATCCTGGCTCAGGACGA...AGTCGTAACAAGGTAGC	13858
[98]	1544	ATCCTGGCTCAGGACGA...GGTGGATCACCTCCTTC	13860
[99]	1482	GGACGAACGCTGGCGGC...GCCGATGATTGGGGTGA	13861
[100]	1485	GACGAACGCTGGCGGCG...GAAGTCGTAACAAGGTA	13862

```
R> length(s)
```

```
[1] 100
```

```
R> s[[1]]
```

```
1521-letter "DNASTring" instance
seq: TTTGATCCTGGCTCAGGACGAACGCTGGCGG...TGTACCGGAAGGTGCGGCTGGATCACCTCCT
```

```
R> substr(s[[1]], 1, 50)
```

```
50-letter "DNASTring" instance
seq: TTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATGCATGCAAG
```

Sequences in the database can also be filtered using classification information. For example, we can get all sequences of the genus name “Desulfosporomusa” by specifying rank and name.

```
R> s <- getSequences(db, rank="Genus", name="Desulfosporomusa")
R> s
```

```
A DNASTringSet instance of length 7
      width seq                      names
[1]  1498 TNGAGAGTTTGATCCTGG...TGGGGCCGATGATCGGGG 13834
[2]  1481 CTGGCGGCGTGCCTAACA...ATTGGGGTGAAGTCGTAA 13836
[3]  1510 GACGAACGCTGGCGGCGT...AGCCGTATCGGAAGGTGC 13839
[4]  1503 ACGCTGGCGGCGTGCCTA...GGTAGCCGTATCGGAAGG 13844
[5]  1503 ACGCTGGCGGCGTGCCTA...GGTAGCCGTATCGGAAGG 13845
[6]  1429 ACGCTGGCGGCGTGCCTA...GAAGCCGGTGGGGTAACC 13846
[7]  1504 ACGCTGGCGGCGTGCCTA...GGTAGCCGTATCGGAAGG 13847
```

To obtain a single sequence, `getSequences` can be used with rank equal to “id” and supplying the sequence’s greengenes ID as the name.

```
R> s <- getSequences(db, rank="id", name="1250")
R> s
```

```
A DNASTringSet instance of length 1
      width seq                      names
[1]  1521 TTTGATCCTGGCTCAGGA...GCGGCTGGATCACCTCCT 1250
```

The database also stores a classification hierarchy. We can obtain the classification hierarchy used in the database with `getTaxonomyNames()`.

```
R> getTaxonomyNames(db)

[1] "Kingdom" "Phylum" "Class" "Order" "Family" "Genus"
[7] "Species" "Otu" "Org_name" "Id"
```

To obtain all unique names stored in the database for a given rank we can use `getRank()`.

```
R> getRank(db, rank="Order")

[1] "Thermoanaerobacterales" "Clostridiales"
```

The 100 sequences in our example data base contain organisms from different orders. We can obtain the rank name for each sequence individually by using `all=TRUE` or count how many sequences we have for each genus using `count=TRUE`.

```
R> getRank(db, rank="Genus", all=TRUE)
```

[1] Coprothermobacter	Desulfotomaculum
[3] Desulfotomaculum	Desulfotomaculum
[5] Desulfotomaculum	Desulfotomaculum
[7] Desulfotomaculum	Desulfotomaculum
[9] Desulfotomaculum	Pelotomaculum
[11] Desulfotomaculum	Desulfotomaculum
[13] Pelotomaculum	Desulfotomaculum
[15] Desulfotomaculum	Desulfotomaculum
[17] Desulfotomaculum	Pelotomaculum
[19] Desulfotomaculum	Desulfotomaculum
[21] Desulfotomaculum	Desulfotomaculum
[23] Desulfotomaculum	Desulfotomaculum
[25] Pelotomaculum	Syntrophomonas
[27] Syntrophomonas	Syntrophomonas
[29] Syntrophomonas	Syntrophomonas
[31] unknown	Syntrophomonas
[33] Moorella	Moorella
[35] Moorella	Moorella
[37] Thermacetogenium	Thermaerobacter
[39] Carboxydotherrmus	Carboxydotherrmus
[41] Thermoanaerobacterium	Thermoanaerobacterium
[43] Thermoanaerobacterium	Thermoanaerobacterium
[45] Thermoanaerobacterium	Thermoanaerobacterium
[47] Thermoanaerobacterium	Thermoanaerobacterium
[49] Thermoanaerobacter	Thermoanaerobacter
[51] Thermoanaerobacter	Thermoanaerobacter
[53] Thermoanaerobacter	Thermoanaerobacter
[55] Thermoanaerobacter	Thermoanaerobacter
[57] Thermoanaerobacter	Thermoanaerobacter
[59] Selenomonas	Selenomonas
[61] Selenomonas	Selenomonas
[63] Selenomonas	Mitsuokella
[65] Selenomonas	Selenomonas
[67] Selenomonas	unknown
[69] Selenomonas	Veillonella
[71] Veillonella	Veillonella
[73] Veillonella	Veillonella
[75] Dialister	Dialister
[77] Dialister	Desulfosporomusa
[79] Desulfosporomusa	unknown
[81] unknown	Desulfosporomusa

```

[83] Thermosinus          Thermosinus
[85] unknown              Desulfosporomusa
[87] Desulfosporomusa     Desulfosporomusa
[89] Desulfosporomusa     unknown
[91] unknown              Acidaminococcus
[93] Acidaminococcus      unknown
[95] unknown              unknown
[97] Phascolarctobacterium Phascolarctobacterium
[99] unknown              unknown
19 Levels: Acidaminococcus Carboxydotherrmus ... Veillonella

```

```
R> getRank(db, rank="Genus", count=TRUE)
```

Desulfotomaculum	unknown	Thermoanaerobacter
20	12	10
Selenomonas	Thermoanaerobacterium	Desulfosporomusa
9	8	7
Syntrophomonas	Veillonella	Moorella
6	5	4
Pelotomaculum	Dialister	Acidaminococcus
4	3	2
Carboxydotherrmus	Phascolarctobacterium	Thermosinus
2	2	2
Coprothermobacter	Mitsuokella	Thermacetogenium
1	1	1
Thermaerobacter		
1		

This information can be easily turned into a barplot showing the abundance of different orders in the data database (see Figure 3).

```

R> oldpar <- par(mar=c(12,5,5,5)) ### make space for labels
R> barplot(sort(
+   getRank(db, rank="Genus", count=TRUE, removeUnknown=TRUE),
+   decreasing=TRUE), las=2)
R> par(oldpar)

```

Filtering also works for `getRank()`. For example, we can find the genera within the order “Thermoanaerobacterales”.

```

R> getRank(db, rank="Genus",
+   whereRank="Order", whereName="Thermo")

```

```

[1] "Coprothermobacter" "Moorella"          "Thermacetogenium"
[4] "Carboxydotherrmus" "Thermoanaerobacter"

```

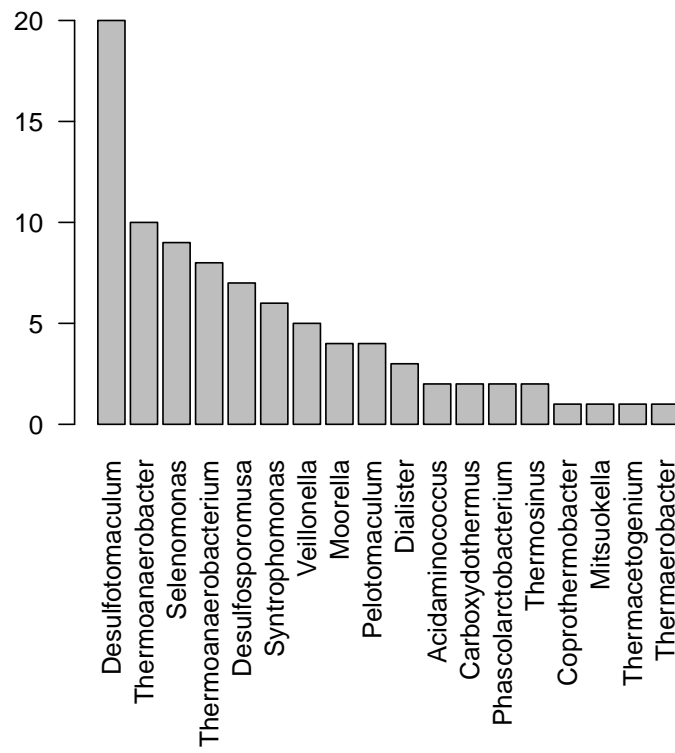


Figure 2: Abundance of different orders in the database.

Note that partial matching is performed from “Thermo” to “Thermoanaerobacterales.” Partial matching is available for ranks and names in most operations in **BioTools**.

We can also get the complete classification hierarchy for different ranks down to individual sequences. In the following we get the classification hierarchy for genus *Thermaerobacter*, then all orders matching *Therm* and then for a list of names.

```
R> getHierarchy(db, rank="Genus", name="Thermaerobacter")
```

Kingdom	Phylum	Class
"Bacteria"	"Firmicutes"	"Clostridia"
Order	Family	Genus
"Clostridiales"	"Sulfobacillaceae"	"Thermaerobacter"
Species	Otu	Org_name
NA	NA	NA
Id		
NA		

```
R> getHierarchy(db, rank="Genus", name="Therm")
```

	Kingdom	Phylum	Class	Order	
[1,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Thermoanaerobacterales"	
[2,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Clostridiales"	
[3,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Clostridiales"	
[4,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Thermoanaerobacterales"	
[5,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Clostridiales"	
	Family				
[1,]	"Thermoanaerobacteraceae"				
[2,]	"Sulfobacillaceae"				
[3,]	"Thermoanaerobacterales Family III. Incertae Sedis"				
[4,]	"Thermoanaerobacteraceae"				
[5,]	"Veillonellaceae"				
	Genus	Species	Otu	Org_name	Id
[1,]	"Thermacetogenium"	NA	NA	NA	NA
[2,]	"Thermaerobacter"	NA	NA	NA	NA
[3,]	"Thermoanaerobacterium"	NA	NA	NA	NA
[4,]	"Thermoanaerobacter"	NA	NA	NA	NA
[5,]	"Thermosinus"	NA	NA	NA	NA

```
R> getHierarchy(db, rank="Genus", name=c("Acid", "Thermo"))
```

	Kingdom	Phylum	Class	Order
[1,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Clostridiales"
[2,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Clostridiales"
[3,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Thermoanaerobacterales"
[4,]	"Bacteria"	"Firmicutes"	"Clostridia"	"Clostridiales"
	Family			


```

[1,] "Veillonellaceae"
[2,] "Thermoanaerobacterales Family III. Incertae Sedis"
[3,] "Thermoanaerobacteraceae"
[4,] "Veillonellaceae"
      Genus      Species Otu Org_name Id
[1,] "Acidaminococcus"   NA    NA   NA    NA
[2,] "Thermoanaerobacterium" NA    NA   NA    NA
[3,] "Thermoanaerobacter"  NA    NA   NA    NA
[4,] "Thermosinus"        NA    NA   NA    NA

```

To get individual sequences we can use again the unique sequence id.

```
R> getHierarchy(db, rank="id", name="1250")
```

```

      Kingdom
      "Bacteria"
      Phylum
      "Firmicutes"
      Class
      "Clostridia"
      Order
      "Thermoanaerobacterales"
      Family
      "Thermodesulfobiaceae"
      Genus
      "Coprothermobacter"
      Species
      "unknown"
      Otu
      "otu_2281"
      Org_name
      "X69335.1Coprothermobacterproteolyticustr.ATCC35245"
      Id
      "1250"

```

Finally, we can close a GenDB after we are done working with it. The database can later be reopened using `openGenDB()`.

```
R> closeGenDB(db)
```

To permanently remove the database we need to delete the file (for SQLite databases) or remove the database using the administrative tool for the database management system.

```
R> unlink("example.sqlite")
```

FIXME: Is there a purge function in DBI to do this?

3. Multiple Sequence Alignment

3.1. clustalw

```
R> dna <- readDNASTringSet(system.file("examples/DNA_example.fasta",
+   package="BioTools"))
R> dna
```

```
A DNASTringSet instance of length 5
      width seq                                     names
[1]  1481 AGAGTTTGATCCTGGCTC...AGTCGTAACAAGGTAACC 1675 AB015560.1 d...
[2]  1404 GCTGGCGGCAGGCCTAAC...TAAGGTCAGCGACTGGGG 4399 D14432.1 Rho...
[3]  1426 GGAATGCTNAACACATGC...GGTAGCCGTAGGGGAACC 4403 X72908.1 Ros...
[4]  1362 GCTGGCGGAATGCTTAAC...TAGGTGTCTAGGCTAACC 4404 AF173825.1 A...
[5]  1458 AGAGTTTGATTATGGCTC...TCGTAACAAGGTAACCGT 4411 Y07647.2 Dre...
```

```
R> al <- clustal(dna)
R> al
```

```
DNAMultipleAlignment with 5 rows and 1500 columns
      aln                                             names
[1] -----...AAGGTAGCCGTAGGGGAACC 4403
[2] -----...----- 4404
[3] AGAGTTTGATTATGGCTCAGA...AAGGTAACCGT----- 4411
[4] -----...----- 4399
[5] AGAGTTTGATCCTGGCTCAGA...AAGGTAACC----- 1675
```

```
R> ### inspect alignment
R> detail(al)
```

```
R> plot(al, 1, 20)
```

```
R> rna <- readRNASTringSet(system.file("examples/RNA_example.fasta",
+   package="BioTools"))
R> rna
```

```
A RNASTringSet instance of length 5
      width seq                                     names
[1]  1481 AGAGUUUGAUCCUGGCUC...AGUCGUAACAAGGUAACC 1675 AB015560.1 d...
[2]  1404 GCUGGCGGCAGGCCUAAC...UAAGGUCAGCGACUGGGG 4399 D14432.1 Rho...
[3]  1426 GGAAUGCUNAACACAUGC...GGUAGCCGUAGGGGAACC 4403 X72908.1 Ros...
[4]  1362 GCUGGCGGAUUGCUUAAC...UAGGUGUCUAGGCUAACC 4404 AF173825.1 A...
[5]  1458 AGAGUUUGAUUAUGGCUC...UCGUAACAAGGUAACCGU 4411 Y07647.2 Dre...
```

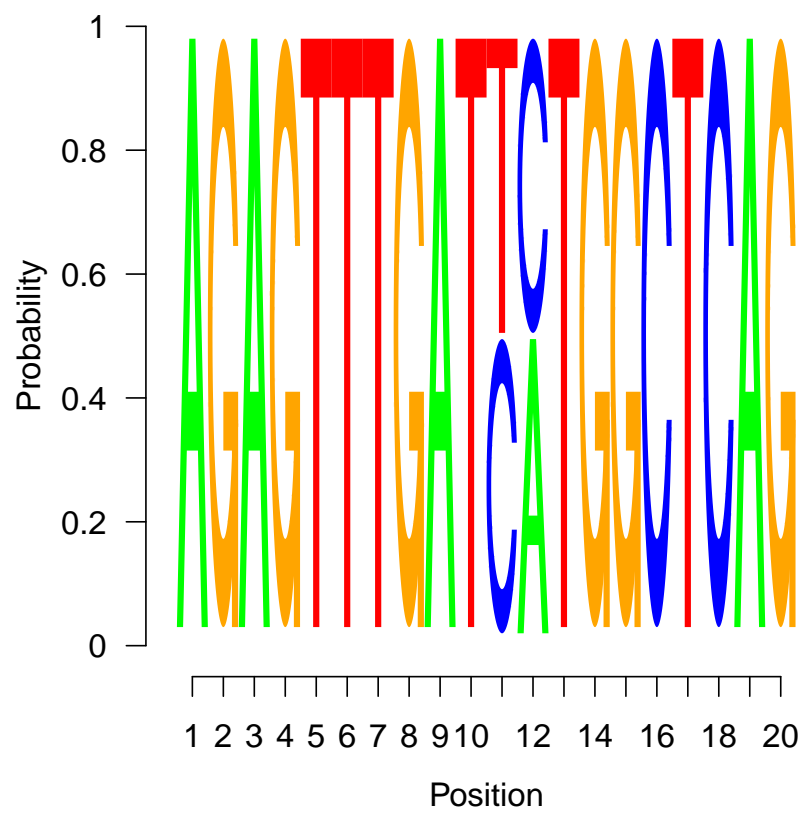


Figure 3: Sequence logo of alignment.

```
R> al <- clustal(rna)
```

```
R> al
```

```
RNAMultipleAlignment with 5 rows and 1500 columns
```

	aln	names
[1]	-----...AAGGUAGCCGUAGGGGAACC	4403
[2]	-----...-----	4404
[3]	AGAGUUUGAUUAUGGCUCAGA...AAGGUAACCGU-----	4411
[4]	-----...-----	4399
[5]	AGAGUUUGAUCCUGGCUCAGA...AAGGUAACC-----	1675

```
R> aa <- readAAStringSet(system.file("examples/Protein_example.fasta",
+ package="BioTools"))
```

```
R> aa
```

```
A AAStringSet instance of length 5
```

	width	seq	names
[1]	170	MKKSRRRIWIFGLLFSIW...DVYYLEAPFFQGRKCGGT	gi 340754543 ref ...
[2]	233	MYIIWKLLFFKGENVVEH...KEEEVISVDDILKKRRE	gi 340754544 ref ...
[3]	326	MKRSLSGIQPSGILHLGN...KKVQEAKEIVGLLGNIYR	gi 340754545 ref ...
[4]	317	MKYYSGVDLGGTNTKIGL...VLGNEAGILGAAALFMLS	gi 340754546 ref ...
[5]	337	MKKMGIILGALVLAAGLV...IVLVPSIGIDKENVAEYK	gi 340754547 ref ...

```
R> al <- clustal(aa)
```

```
R> al
```

```
AAMultipleAlignment with 5 rows and 358 columns
```

	aln	names
[1]	---MKKSRRRIWIFGLLFSIW...-----	gi 340754543 ref ...
[2]	---MYIIWKLLFFKGENVVEH...-----	gi 340754544 ref ...
[3]	MKKMGIILGALVLAAGLVGCG...DKENVAEYK-----	gi 340754547 ref ...
[4]	---MKRSLSGIQPSGILHLGN...ASKKVQEAKEIVGLLGNIYR	gi 340754545 ref ...
[5]	----MKYYSGVDLGGTNTKIG...-----	gi 340754546 ref ...

3.2. kalign

```
R> dna <- readDNStringSet(system.file("examples/DNA_example.fasta",
+ package="BioTools"))
```

```
R> dna
```

```
A DNStringSet instance of length 5
```

	width	seq	names
[1]	1481	AGAGTTTGATCCTGGCTC...AGTCGTAACAAGGTAACC	1675 AB015560.1 d...
[2]	1404	GCTGGCGGCAGGCCTAAC...TAAGGTCAGCGACTGGGG	4399 D14432.1 Rho...
[3]	1426	GGAATGCTNAACACATGC...GGTAGCCGTAGGGGAACC	4403 X72908.1 Ros...
[4]	1362	GCTGGCGGAATGCTTAAC...TAGGTGTCTAGGCTAACC	4404 AF173825.1 A...
[5]	1458	AGAGTTTGATTATGGCTC...TCGTAACAAGGTAACCGT	4411 Y07647.2 Dre...

```
R> ### align the sequences
R> al <- kalign(dna)
R> al
```

DNAMultipleAlignment with 5 rows and 1502 columns

	aln	names
[1]	AGAGTTTGATCCTGGCTCAGA...-----CAAGGTAAC--C	1675 AB015560.1 d...
[2]	G-----...-----TGGG-----G	4399 D14432.1 Rho...
[3]	G-----...GGTAGCCGTAGGGGAAC--C	4403 X72908.1 Ros...
[4]	G-----...-----TAGGCTAAC--C	4404 AF173825.1 A...
[5]	AGAGTTTGATTATGGCTCAGA...-----CAAGGTAACCGT	4411 Y07647.2 Dre...

4. Classification with RDP

4.1. Using the default RDP classifier

Use the default classifier

```
R> seq <- readRNAStringSet(system.file("examples/RNA_example.fasta",
+   package="BioTools"))
R> ## shorten names
R> names(seq) <- sapply(strsplit(names(seq), " "), "[", 1)
R> seq
```

A RNAStringSet instance of length 5

	width	seq	names
[1]	1481	AGAGUUUGAUCCUGGCUC...AGUCGUAACAAGGUAACC	1675
[2]	1404	GCUGGCGGCAGGCCUAAC...UAAGGUCAGCGACUGGGG	4399
[3]	1426	GGAAUGCUNAACACAUGC...GGUAGCCGUAGGGGAACC	4403
[4]	1362	GCUGGCGGAAUGCUGUAAC...UAGGUGUCUAGGCUAACC	4404
[5]	1458	AGAGUUUGAUUAUGGCUC...UCGUAACAAGGUAACCGU	4411

```
R> ## use rdp for classification
R> predict(RDP(), seq)
```

	norank	domain	phylum	class
1675	Root	Bacteria	Proteobacteria	Deltaproteobacteria
4399	Root	Bacteria	Proteobacteria	Alphaproteobacteria
4403	Root	Bacteria	Proteobacteria	Alphaproteobacteria
4404	Root	Bacteria	Proteobacteria	Alphaproteobacteria
4411	Root	Bacteria	Proteobacteria	Alphaproteobacteria

	order	family	genus
1675	<NA>	<NA>	<NA>
4399	Rhodospirillales	Rhodospirillaceae	Rhodovibrio
4403	Rhodospirillales	Acetobacteraceae	Roseococcus

```
4404 Rhodospirillales  Acetobacteraceae Roseococcus
4411 Rhodospirillales  Acetobacteraceae      <NA>
```

4.2. Training a custom RDP classifier

Train a custom RDP classifier on new data

```
R> trainingSequences <- readDNASTringSet(
+   system.file("examples/trainingSequences.fasta", package="BioTools"))
R> customRDP <- trainRDP(trainingSequences)
R> customRDP
```

RDPClassifier

Location: /home/hahsler/baR/QuasiAlign/pkg/BioTools/Work/vignette/classifier

```
R> testSequences <- readDNASTringSet(
+   system.file("examples/testSequences.fasta", package="BioTools"))
R> predict(customRDP, testSequences)
```

	rootrank	Kingdom	Phylum	Class	Order
13811	Root	Bacteria	Firmicutes	Clostridia	Clostridiales
13813	Root	Bacteria	Firmicutes	Clostridia	Clostridiales
13678	Root	Bacteria	Firmicutes	Clostridia	Clostridiales
13755	Root	Bacteria	Firmicutes	Clostridia	Clostridiales
13661	Root	Bacteria	Firmicutes	Clostridia	Clostridiales
					Family
13811				Veillonellaceae	
13813				Veillonellaceae	
13678				Peptococcaceae	
13755	Thermoanaerobacterales	Family III.	Incertae	Sedis	
13661				Peptococcaceae	
			Genus		
13811		Selenomonas			
13813		Selenomonas			
13678		Desulfotomaculum			
13755		Thermoanaerobacterium			
13661		Desulfotomaculum			

```
R> ## clean up
R> removeRDP(customRDP)
```

5. Sequence Retrieval with BLAST

```

R> seq <- readRNAStringSet(system.file("examples/RNA_example.fasta",
+   package="BioTools"))
R> ## shorten names
R> names(seq) <- sapply(strsplit(names(seq), " "), "[", 1)
R> seq
R> ## run BLAST (I have the NCBI database downloaded to ~/tmp/blast)
R> cl <- BLAST(seq, db="~/tmp/blast/refseq_rna")
R> cl[1:5, ]

```

6. Conclusion

Acknowledgments

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References

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). "Basic local alignment search tool." *Journal of Molecular Biology*, **215**(3), 403–410. ISSN 0022-2836.
- Edgar R (2004a). "MUSCLE: a multiple sequence alignment method with reduced time and space complexity." *BMC Bioinformatics*, **5**(1), 113+. ISSN 1471-2105.
- Edgar RC (2004b). "Muscle: multiple sequence alignment with high accuracy and high throughput." *Nucleic Acids Research*, **32**, 1792–1797.
- Katoh K, Misawa K, Kuma K, Miyata T (2002). "MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform." *Nucleic Acids Research*, **30**(14), 3059–3066.
- Larkin M, Blackshields G, Brown N, Chenna R, McGettigan P, McWilliam H, Valentin F, Wallace I, Wilm A, Lopez R, Thompson J, Gibson T, Higgins D (2007). "Clustal W and Clustal X version 2.0." *Bioinformatics*, **23**, 2947–2948. ISSN 1367-4803.
- Lassmann T, Sonnhammer EL (2006). "Kalign, Kalignvu and Mumsa: web servers for multiple sequence alignment." *Nucleic Acids Research*, **34**. ISSN 1362-4962.
- Notredame C, Higgins DG, Heringa J (2000). "T-Coffee: A novel method for fast and accurate multiple sequence alignment." *Journal of Molecular Biology*, **302**(1), 205–217. ISSN 0022-2836.
- Smith TF, Waterman MS (1981). "Identification of common molecular subsequences." *Journal of Molecular Biology*, **147**(1), 195–197. ISSN 0022-2836.

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