

# rRDP: Interface to the RDP Classifier

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## Abstract

This package installs and interfaces the naive Bayesian classifier for 16S rRNA sequences developed by the Ribosomal Database Project (RDP). With this package the classifier trained with the standard training set can be used or a custom classifier can be trained.

*Keywords:* bioinformatics, Bioconductor, Biostrings, sequence classification.

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## 1. Installation and System requirements

rRDP requires the Bioconductor package Biostrings and R to be configured with Java.

```
R> if (!require("BiocManager", quietly = TRUE))
+   install.packages("BiocManager")
R> BiocManager::install("Biostrings")
```

Install rRDP and the database used by the default RDP classifier.

```
R> BiocManager::install("rRDP")
R> BiocManager::install("rRDPData")
```

To configure R for Java, you need to install a Java JDK. On Linux, you can install Open JDK and run in your shell R CMD javareconf.

On Windows, you can install the latest version of the JDK from <https://www.oracle.com/java/technologies/downloads/> and set the JAVA\_HOME environment variable in R using (make sure to use the correct location).

```
R> Sys.setenv(JAVA_HOME = "C:\\Program Files\\Java\\jdk-20")
```

Note the double backslashes (i.e. escaped slashes) used in the path.

## 2. How to cite this package

```
R> citation("rRDP")
```

To cite package ‘rRDP’ in publications use:

```
Hahsler M, Nagar A (2020). "rRDP: Interface to the RDP
Classifier." Bioconductor version: Release (3.17).
doi:10.18129/B9.bioc.rRDP
<https://doi.org/10.18129/B9.bioc.rRDP>, R package version
1.23.1.
```

A BibTeX entry for LaTeX users is

```
@Misc{,
  title = {{rRDP:} Interface to the {RDP} Classifier},
  author = {Michael Hahsler and Annurag Nagar},
  year = {2020},
  doi = {10.18129/B9.bioc.rRDP},
  note = {R package version 1.23.1},
  howpublished = {Bioconductor version: Release (3.17)},
}
```

### 3. Classification with RDP

The RDP classifier was developed by the Ribosomal Database Project which provides various tools and services to the scientific community for data related to 16S rRNA sequences. The classifier uses a Naive Bayesian approach to quickly and accurately classify sequences. The classifier uses 8-mer counts as features [Wang, Garrity, Tiedje, and Cole \(2007\)](#).

#### 3.1. Using the RDP classifier trained with the default training set

RDP is shipped trained with a 16S rRNA training set. The model data is available in the data package **rRDPData**.

For the following example we load some test sequences shipped with the package.

```
R> library(rRDP)
R> seq <- readRNAStringSet(system.file("examples/RNA_example.fasta",
+   package="rRDP"))
R> seq
```

RNAStringSet object of length 5:

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

Note that the name contains the annotation from the FASTA file. In this case the annotation contains the actual classification information and is encoded in Greengenes format. For convenience, we replace the annotation with just the sequence id.

```
R> annotation <- names(seq)
R> names(seq) <- sapply(strsplit(names(seq), " "), "[", 1)
R> seq
```

RNAStringSet object of length 5:

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

Next, we apply RDP with the default training set. Note that the data package **rRDPDate** needs to be installed!

```
R> pred <- predict(rdp(), seq)
R> pred
```

	domain	phylum	class	order
1675	Bacteria	Proteobacteria	Deltaproteobacteria	<NA>
4399	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
4403	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
4404	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
4411	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
		family	genus	
1675		<NA>	<NA>	
4399	Rhodospirillaceae	Rhodovibrio		
4403	Acetobacteraceae	Roseococcus		
4404	Acetobacteraceae	Roseococcus		
4411	Acetobacteraceae		<NA>	

The prediction confidence is supplied as the attribute "confidence".

```
R> attr(pred, "confidence")
```

	domain	phylum	class	order	family	genus
1675	1	0.91	0.91	0.43	0.43	0.42
4399	1	1.00	1.00	1.00	1.00	1.00
4403	1	1.00	1.00	1.00	1.00	1.00
4404	1	1.00	1.00	1.00	1.00	1.00
4411	1	1.00	1.00	1.00	1.00	0.39

To evaluate the classification accuracy we can compare the known classification with the predictions. The known classification was stored in the FASTA file and encoded in Greengenes format. We can decode the annotation using `decode_Greengenes()`.

```
R> actual <- decode_Greengenes(annotation)
```

```
R> actual
```

	Kingdom	Phylum	Class	Order
1	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales
2	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
3	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
4	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
5	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
		Family	Genus	Species
1		Nitrospinaceae	Nitrospina	unknown
2		Rhodospirillaceae	Rhodovibrio	Rhodovibrio salinarum
3		Acetobacteraceae	Roseococcus	unknown
4		Acetobacteraceae	Roseococcus	unknown
5	Acetobacteraceae; Unclassified		unknown	unknown
	Otu			
1	3187			
2	2816			
3	2785			
4	2785			
5	2752			
				Org_name
1				AB015560.1_deep-sea_sediment_clone_BD4-10
2				D14432.1_Rhodovibrio_salinarum_str._NCIMB2243
3	X72908.1_Roseococcus_thiosulfatophilus_str._RB-3_Yurkov_strain_Drews			
4				AF173825.1_Antarctic_clone_LB3-94
5				Y07647.2_Drentse_grassland_soil_clone_vii
	Id			
1	1675			
2	4399			
3	4403			
4	4404			
5	4411			

Now we can compare the prediction with the actual classification by creating a confusion table and calculating the classification accuracy. Here we do this at the Genus level.

```
R> confusionTable(actual, pred, rank="genus")
```

actual	predicted				
	Nitrospina	Rhodovibrio	Roseococcus	unknown	<NA>
Nitrospina	0	0	0	0	1
Rhodovibrio	0	1	0	0	0

Roseococcus	0	0	2	0	0
unknown	0	0	0	0	1
<NA>	0	0	0	0	0

```
R> accuracy(actual, pred, rank="genus")
```

```
[1] 0.6
```

### 3.2. Training a custom RDP classifier

RDP can be trained using `trainRDP()`. We use an example of training data that is shipped with the package.

```
R> trainingSequences <- readDNAStrngSet(
+   system.file("examples/trainingSequences.fasta", package="rRDP"))
R> trainingSequences
```

DNAStrngSet object of length 20:

	width	seq	names
[1]	1384	TAGTGGCGGACGGGTGAG...TCGAATTTGGGTCAAGT	13652 Root;Bacter...
[2]	1386	ATCTCACCTCTCAATAGC...CGAAGGTGGGTTGGTG	13655 Root;Bacter...
[3]	1440	ATCTCACCTCTCAATAGC...GCTGGATCACCTCCTTA	13661 Root;Bacter...
[4]	1421	AATAGCGGCGGACGGGTG...ATCGGAAGGTGCGGCTG	13671 Root;Bacter...
[5]	1439	ATCTCACCTCTCAATANC...GGTGGGCTGGATCACC	13677 Root;Bacter...
...	...	...	...
[16]	1478	TGGCTCAGGACGAACGCT...CGTATCGGAAGGTGCGG	13763 Root;Bacter...
[17]	1507	CCTGGCTCAGGACGAACG...TATCGGAAGGTGCGGCT	13781 Root;Bacter...
[18]	1481	TGGAGAGTTTGATCCTGG...GCAAGGATATAGCCGTC	13797 Root;Bacter...
[19]	1463	CGGCGTGCTTGGACCCAC...GGTCCTAAGGTGGGGGC	13799 Root;Bacter...
[20]	1389	CGAGTGGCAAACGGGTGA...GCAAGGATGCAGCCGTC	13800 Root;Bacter...

Note that the training data needs to have names in a specific RDP format:

```
"<ID> <Kingdom>;<Phylum>;<Class>;<Order>;<Family>;<Genus>"
```

In the following we show the name for the first sequence. We use here `sprintf` to display only the first 65 characters so the it fits into a single line.

```
R> sprintf(names(trainingSequences[1]), fmt="%.65s...")
```

```
[1] "13652 Root;Bacteria;Firmicutes;Clostridia;Clostridiales;Peptococc..."
```

Now, we can train a the classifier. The model is stored in a directory specified by the parameter `dir`.

```
R> customRDP <- trainRDP(trainingSequences, dir = "myRDP")
R> customRDP
```

```
RDPClassifier
```

```
Location: /tmp/RtmpVDR8AE/Rbuild16085d4211dca7/rRDP/vignettes/myRDP
```

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

	domain	Phylum	Class	Order
13811	Firmicutes	Firmicutes	Clostridia	Clostridiales
13813	Firmicutes	Firmicutes	Clostridia	Clostridiales
13678	Firmicutes	Firmicutes	Clostridia	Clostridiales
13755	Firmicutes	Firmicutes	Clostridia	Clostridiales
13661	Firmicutes	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		

Since the custom classifier is stored on disc it can be recalled anytime using `rdp()`.

```
R> customRDP <- rdp(dir = "myRDP")
```

To permanently remove the classifier use `removeRDP()`.

```
R> removeRDP(customRDP)
```

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## References

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007). "Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy." *Applied and environmental microbiology*, **73**(16), 5261–5267.

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