msa

An R Package for Multiple Sequence Alignment

Enrico Bonatesta, Christoph Horejš-Kainrath, and Ulrich Bodenhofer

Institute of Bioinformatics, Johannes Kepler University Linz
Altenberger Str. 69, 4040 Linz, Austria
msa@bioinf.jku.at

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Scope and Purpose of this Document

This document provides a gentle introduction into the `R` package `msa`. Not all features of the `R` package are described in full detail. Such details can be obtained from the documentation enclosed in the `R` package. Further note the following: (1) this is not an introduction to multiple sequence alignment or algorithms for multiple sequence alignment; (2) this is not an introduction to `R` or any of the Bioconductor packages used in this document. If you lack the background for understanding this manual, you first have to read introductory literature on the subjects mentioned above.

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1 Introduction

Multiple sequence alignment is one of the most fundamental tasks in bioinformatics. Algorithms like ClustalW [13], ClustalOmega [12], and MUSCLE [3, 4] are well known and widely used. However, all these algorithms are implemented as stand-alone commmand line programs without any integration into the R/Bioconductor ecosystem. Before the `msa` package, only the `muscle` package has been available in `R`, but no other multiple sequence alignment algorithm, although the `Biostrings` package has provided data types for representing multiple sequence alignments for quite some time. The `msa` package aims to close that gap by providing a unified R interface to the multiple sequence alignment algorithms ClustalW, ClustalOmega, and MUSCLE. The package requires no additional software packages and runs on all major platforms. Moreover, the `msa` package provides an R interface to the powerful LaTeX package TeXshade [1] which allows for a highly customizable plots of multiple sequence alignments. Unless some very special features of TeXshade are required, users can pretty-print multiple sequence alignments without the need to know the details of LaTeX or TeXshade.

2 Installation

The `msa` `R` package (current version: 1.9.0) is available via Bioconductor. The simplest way to install the package is the following:

```
source("http://www.bioconductor.org/biocLite.R")
biocLite("msa")
```

To test the installation of the `msa` package, enter

```
library(msa)
```

in your `R` session. If this command terminates without any error message or warning, you can be sure that the `msa` package has been installed successfully. If so, the `msa` package is ready for use now and you can start performing multiple sequence alignments.

To make use of all functionalities of `msaPrettyPrint()`, a TeX/LaTeX system [5] must be installed. To make use of LaTeX code created by `msaPrettyPrint()` or to use the output of `msaPrettyPrint()` in Sweave [6] or knitr [15] documents, the LaTeX package TeXshade (file `texshade.sty`) [1] must be accessible to the LaTeX system too. The file `texshade.sty` is shipped with the `msa` package. To determine where the file is located, enter the following command in your `R` session:

```
system.file("tex", "texshade.sty", package="msa")
```

Alternatively, TeXshade can be installed directly from the Comprehensive TeX Archive Network (CTAN).[1]

[1] `https://www.ctan.org/pkg/texshade`

3 msa for the Impatient

In order to illustrate the basic workflow, this section presents a simple example with default settings and without going into the details of each step. Let us first load amino acid sequences from one of the example files that are supplied with the msa package:

```
mySequenceFile <- system.file("examples", "exampleAA.fasta", package="msa")
mySequences <- readAAStringSet(mySequenceFile)
mySequences

##   A AAStringSet instance of length 9
##     width seq                                  names
## [1]   452 MSTAVLENPGLGRKLS...NSEIGILCSALQKIK PH4H_Homo_sapiens
## [2]   453 MAAVVLENGVLSRKLS...SEVGILCNALQKIKS PH4H_Rattus_norve...
## [3]   453 MAAVVLENGVLSRKLS...SEVGILCHALQKIKS PH4H_Mus_musculus
## [4]   297 MNDRADFVVPDITTRK...LNAGDRQGWADTEDV PH4H_Chromobacter...
## [5]   262 MKTTQYVARQPDDNGF...RLGLHAPLFPPKQAA PH4H_Pseudomonas_...
## [6]   451 MSALVLESRALGRKLS...SSEVEILCSALQKLK PH4H_Bos_taurus
## [7]   313 MAIATPTSAAPTPAPA...LNAGTREGWADTADI PH4H_Ralstonia_so...
## [8]   294 MSGDGLSNGPPPGARP...AYATAGGRLAGAAAG PH4H_Caulobacter_...
## [9]   275 MSVAEYARDCAAQGLR...VARRKDQKALDPATV PH4H_Rhizobium_loti
```

Now that we have loaded the sequences, we can run the msa() function which, by default, runs ClustalW with default parameters:

```
myFirstAlignment <- msa(mySequences)

## use default substitution matrix

myFirstAlignment

## CLUSTAL 2.1
##
## Call:
##    msa(mySequences)
##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln                                       names
## [1] MAAVVLENGVLSRKLSDF...SINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] MAAVVLENGVLSRKLSDF...SINSEVGILCHALQKIKS PH4H_Mus_musculus
## [3] MSTAVLENPGLGRKLSDF...SINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [4] MSALVLESRALGRKLSDF...SISSEVEILCSALQKLK- PH4H_Bos_taurus
## [5] ------------------...GWADTEDV---------- PH4H_Chromobacter...
## [6] ------------------...GWADTADI---------- PH4H_Ralstonia_so...
## [7] ------------------...AYATAGGRLAGAAAG--- PH4H_Caulobacter_...
```

```
## [8] ------------------...------------------ PH4H_Pseudomonas_...
## [9] ------------------...------------------ PH4H_Rhizobium_loti
## Con ------------------...???????IL??A???--- Consensus
```

Obviously, the default printing function shortens the alignment for the sake of compact output. The `print()` function provided by the `msa` package provides some ways for customizing the output, such as, showing the entire alignment split over multiple blocks of sub-sequences:

```
print(myFirstAlignment, show="complete")

##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln (1..39)                              names
## [1] MAAVVLENGVLSRKLSDFGQETSYIEDNSNQNGAISLIF PH4H_Rattus_norve...
## [2] MAAVVLENGVLSRKLSDFGQETSYIEDNSNQNGAVSLIF PH4H_Mus_musculus
## [3] MSTAVLENPGLGRKLSDFGQETSYIEDNCNQNGAISLIF PH4H_Homo_sapiens
## [4] MSALVLESRALGRKLSDFGQETSYIEGNSDQN-AVSLIF PH4H_Bos_taurus
## [5] ---------------------------------------- PH4H_Chromobacter...
## [6] ---------------------------------------- PH4H_Ralstonia_so...
## [7] ---------------------------------------- PH4H_Caulobacter_...
## [8] ---------------------------------------- PH4H_Pseudomonas_...
## [9] ---------------------------------------- PH4H_Rhizobium_loti
## Con ---------------------------------------- Consensus
##
##     aln (40..78)                             names
## [1] SLKEEVGALAKVLRLFEENDINLTHIESRPSRLNKDEYE PH4H_Rattus_norve...
## [2] SLKEEVGALAKVLRLFEENEINLTHIESRPSRLNKDEYE PH4H_Mus_musculus
## [3] SLKEEVGALAKVLRLFEENDVNLTHIESRPSRLKKDEYE PH4H_Homo_sapiens
## [4] SLKEEVGALARVLRLFEENDINLTHIESRPSRLRKDEYE PH4H_Bos_taurus
## [5] ---------------------------------------- PH4H_Chromobacter...
## [6] ---------------------------------------- PH4H_Ralstonia_so...
## [7] ---------------------------------------- PH4H_Caulobacter_...
## [8] ---------------------------------------- PH4H_Pseudomonas_...
## [9] ---------------------------------------- PH4H_Rhizobium_loti
## Con ---------------------------------------- Consensus
##
##     aln (79..117)                            names
## [1] FFTYLDKRTKPVLGSIIKSLRNDIGATVHELSRDKEKNT PH4H_Rattus_norve...
## [2] FFTYLDKRSKPVLGSIIKSLRNDIGATVHELSRDKEKNT PH4H_Mus_musculus
## [3] FFTHLDKRSLPALTNIIKILRHDIGATVHELSRDKKKDT PH4H_Homo_sapiens
## [4] FFTNLDQRSVPALANIIKILRHDIGATVHELSRDKKKDT PH4H_Bos_taurus
## [5] ---------------------------------------- PH4H_Chromobacter...
## [6] ---------------------------------------- PH4H_Ralstonia_so...
## [7] ---------------------------------------- PH4H_Caulobacter_...
## [8] ---------------------------------------- PH4H_Pseudomonas_...
## [9] ---------------------------------------- PH4H_Rhizobium_loti
```

```
## Con ---------------------------------------- Consensus
##
##     aln (118..156)                           names
## [1] VPWFPRTIQELDRFANQILSYGAELDADHPGFKDPVYRA PH4H_Rattus_norve...
## [2] VPWFPRTIQELDRFANQILSYGAELDADHPGFKDPVYRA PH4H_Mus_musculus
## [3] VPWFPRTIQELDRFANQILSYGAELDADHPGFKDPVYRA PH4H_Homo_sapiens
## [4] VPWFPRTIQELDNFANQVLSYGAELDADHPGFKDPVYRA PH4H_Bos_taurus
## [5] ---------------MNDRADFVVPD-----ITTRKNVG PH4H_Chromobacter...
## [6] ----------MAIATPTSAAPTPAPAGFTGTLTDKLREQ PH4H_Ralstonia_so...
## [7] ---------------MSG---------------DGLSNG PH4H_Caulobacter_...
## [8] ---------------------------------MKTTQY PH4H_Pseudomonas_...
## [9] ---------------MSVAEYAR----------DCAAQG PH4H_Rhizobium_loti
## Con ----------??????????Y????D???????D????? Consensus
##
##     aln (157..195)                           names
## [1] RRKQFADIAYNYRHGQPIPRVEYTEEEKQTWGTVFRTLK PH4H_Rattus_norve...
## [2] RRKQFADIAYNYRHGQPIPRVEYTEEERKTWGTVFRTLK PH4H_Mus_musculus
## [3] RRKQFADIAYNYRHGQPIPRVEYMEEEKKTWGTVFKTLK PH4H_Homo_sapiens
## [4] RRKQFADIAYNYRHGQPIPRVEYTEEEKKTWGTVFRTLK PH4H_Bos_taurus
## [5] LSHDAN------DFTLPQPLDRYSAEDHATWATLYQRQC PH4H_Chromobacter...
## [6] FAEGLDGQTLRPDFTMEQPVHRYTAADHATWRTLYDRQE PH4H_Ralstonia_so...
## [7] PPPGAR-----PDWTIDQGWETYTQAEHDVWITLYERQT PH4H_Caulobacter_...
## [8] VARQPD----------DNGFIHYPETEHQVWNTLITRQL PH4H_Pseudomonas_...
## [9] LRGDYS--VCRADFTVAQDYD-YSDEEQAVWRTLCDRQT PH4H_Rhizobium_loti
## Con ?R?Q???????????P?P???YTEEE??TW?TL??RQ? Consensus
##
##     aln (196..234)                           names
## [1] ALYKTHACYEHNHIFPLLEKYCGFREDNIPQLEDVSQFL PH4H_Rattus_norve...
## [2] ALYKTHACYEHNHIFPLLEKYCGFREDNIPQLEDVSQFL PH4H_Mus_musculus
## [3] SLYKTHACYEYNHIFPLLEKYCGFHEDNIPQLEDVSQFL PH4H_Homo_sapiens
## [4] SLYKTHACYEHNHIFPLLEKYCGFREDNIPQLEEVSQFL PH4H_Bos_taurus
## [5] KLLPGRACDEFMEGL----ERLEVDADRVPDFNKLNQKL PH4H_Chromobacter...
## [6] ALLPGRACDEFLQGL----STLGMSREGVPSFDRLNETL PH4H_Ralstonia_so...
## [7] DMLHGRACDEFMRGL----DALDLHRSGIPDFARINEEL PH4H_Caulobacter_...
## [8] KVIEGRACQEYLDGI----EQLGLPHERIPQLDEINRVL PH4H_Pseudomonas_...
## [9] KLTRKLAHHSYLDGV----EKLGL-LDRIPDFEDVSTKL PH4H_Rhizobium_loti
## Con ?L????AC?E???G?----??LG???D?IPQLE?VSQ?L Consensus
##
##     aln (235..273)                           names
## [1] QTCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Rattus_norve...
## [2] QTCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Mus_musculus
## [3] QTCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Homo_sapiens
## [4] QSCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Bos_taurus
## [5] MAATGWKIVAVPGLIPDDVFFEHLANRRFPVTWWLREPH PH4H_Chromobacter...
## [6] MRATGWQIVAVPGLVPDEVFFEHLANRRFPASWWMRRPD PH4H_Ralstonia_so...
## [7] KRLTGWTVVAVPGLVPDDVFFDHLANRRFPAGQFIRKPH PH4H_Caulobacter_...
```

```
## [8] QATTGWRVARVPALIPFQTFFELLASQQFPVATFIRTPE PH4H_Pseudomonas_...
## [9] RKLTGWEIIAVPGLIPAAPFFDHLANRRFPVTNWLRTRQ PH4H_Rhizobium_loti
## Con Q??TGWR???VPGL?P???FF??LA?R?FP?TQ?IR??? Consensus
##
##     aln (274..312)                          names
## [1] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Rattus_norve...
## [2] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Mus_musculus
## [3] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Homo_sapiens
## [4] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Bos_taurus
## [5] QLDYLQEPDVFHDLFGHVPLLINPVFADYLEAYGKGGVK PH4H_Chromobacter...
## [6] QLDYLQEPDGFHDIFGHVPLLINPVFADYMQAYGQGGLK PH4H_Ralstonia_so...
## [7] ELDYLQEPDIFHDVFGHVPMLTDPVFADYMQAYGEGGRR PH4H_Caulobacter_...
## [8] ELDYLQEPDIFHEIFGHCPLLTNPWFAEFTHTYGKLGLK PH4H_Pseudomonas_...
## [9] ELDYIVEPDMFHDFFGHVPVLSQPVFADFMQMYGKKAGD PH4H_Rhizobium_loti
## Con ?LDY??EPDIFHELFGHVPLLSDP?FA?F?Q?YG?LA?? Consensus
##
##     aln (313..351)                          names
## [1] GAPDEYIEKLATIYWFTVEFGLCKEG-DSIKAYGAGLLS PH4H_Rattus_norve...
## [2] GAPDEYIEKLATIYWFTVEFGLCKEG-DSIKAYGAGLLS PH4H_Mus_musculus
## [3] GAPDEYIEKLATIYWFTVEFGLCKQG-DSIKAYGAGLLS PH4H_Homo_sapiens
## [4] GAPDEYIEKLATIYWFTVEFGLCKQG-DSIKAYGAGLLS PH4H_Bos_taurus
## [5] AKALGALPMLARLYWYTVEFGLINTP-AGMRIYGAGILS PH4H_Chromobacter...
## [6] AARLGALDMLARLYWYTVEFGLIRTP-AGLRIYGAGIVS PH4H_Ralstonia_so...
## [7] ALGLGRLANLARLYWYTVEFGLMNTP-AGLRIYGAGIVS PH4H_Caulobacter_...
## [8] ASKE-ERVFLARLYWMTIEFGLVETD-QGKRIYGGGILS PH4H_Pseudomonas_...
## [9] IIALGGDEMITRLYWYTAEYGLVQEAGQPLKAFGAGLMS PH4H_Rhizobium_loti
## Con ?A?????E?LARLYW?TVEFGL????-???KAYGAGLLS Consensus
##
##     aln (352..390)                          names
## [1] SFGELQYCLSD-KPKLLPLELEKTACQEYSVTEFQPLYY PH4H_Rattus_norve...
## [2] SFGELQYCLSD-KPKLLPLELEKTACQEYTVTEFQPLYY PH4H_Mus_musculus
## [3] SFGELQYCLSE-KPKLLPLELEKTAIQNYTVTEFQPLYY PH4H_Homo_sapiens
## [4] SFGELQYCLSD-KPKLLPLELEKTAVQEYTITEFQPLYY PH4H_Bos_taurus
## [5] SKSESIYCLDSASPNRVGFDLMRIMNTRYRIDTFQKTYF PH4H_Chromobacter...
## [6] SKSESVYALDSASPNRIGFDVHRIMRTRYRIDTFQKTYF PH4H_Ralstonia_so...
## [7] SRTESIFALDDPSPNRIGFDLERVMRTLYRIDDFQQVYF PH4H_Caulobacter_...
## [8] SPKETVYSLSD-EPLHQAFNPLEAMRTPYRIDILQPLYF PH4H_Pseudomonas_...
## [9] SFTELQFAVEGKDAHHVPFDLETVMRTGYEIDKFQRAYF PH4H_Rhizobium_loti
## Con SF?ELQYCLSD-?P???PF?LE??M?T?Y?ID?FQPLYF Consensus
##
##     aln (391..429)                          names
## [1] VAESFSDAKEKVRTFAATIPRPFSVRYDPYTQRVEVLDN PH4H_Rattus_norve...
## [2] VAESFNDAKEKVRTFAATIPRPFSVRYDPYTQRVEVLDN PH4H_Mus_musculus
## [3] VAESFNDAKEKVRNFAATIPRPFSVRYDPYTQRIEVLDN PH4H_Homo_sapiens
## [4] VAESFNDAKEKVRNFAATIPRPFSVHYDPYTQRIEVLDN PH4H_Bos_taurus
## [5] VIDSFKQLFDATA-PDFAPLYLQLADAQPWGAGDVAPDD PH4H_Chromobacter...
```

```
## [6] VIDSFEQLFDATR-PDFTPLYEALGTLPTFGAGDVVDGD PH4H_Ralstonia_so...
## [7] VIDSIQTLQEVTL-RDFGAIYERLASVSDIGVAEIVPGD PH4H_Caulobacter_...
## [8] VLPDLKRLFQLAQ-EDIMALVHEAMRLG-LHAPLFPPKQ PH4H_Pseudomonas_...
## [9] VLPSFDALRDAFQTADFEAIVARRKDQKALDPATV---- PH4H_Rhizobium_loti
## Con V??SF??L?E??R??D?T??????????P??????V?D? Consensus
##
##     aln (430..456)              names
## [1] TQQLKILADSINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] TQQLKILADSINSEVGILCHALQKIKS PH4H_Mus_musculus
## [3] TQQLKILADSINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [4] TQQLKILADSISSEVEILCSALQKLK- PH4H_Bos_taurus
## [5] LVLNAGDRQGWADTEDV---------- PH4H_Chromobacter...
## [6] AVLNAGTREGWADTADI---------- PH4H_Ralstonia_so...
## [7] AVLTRGT-QAYATAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8] AA------------------------- PH4H_Pseudomonas_...
## [9] ---------------------------- PH4H_Rhizobium_loti
## Con ??????????????IL??A???--- Consensus
```

The `msa` package additionally offers the function `msaPrettyPrint()` which allows for pretty-printing multiple alignments using the LaTeX package TeXshade. As an example, the following `R` code creates a PDF file `myfirstAlignment.pdf` which is shown in Figure 1:

```
msaPrettyPrint(myFirstAlignment, output="pdf", showNames="none",
               showLogo="none", askForOverwrite=FALSE, verbose=FALSE)
```

In the above call to `msaPrettyPrint()`, the printing of sequence names has been suppressed by `showNames="none"`. The settings `askForOverwrite=FALSE` and `verbose=FALSE` are necessary for building this vignette, but, in an interactive `R` session, they are not necessary.

Almost needless to say, the file names created by `msaPrettyPrint()` are customizable. By default, the name of the argument is taken as file name. More importantly, the actual output of `msaPrettyPrint()` is highly customizable, too. For more details, see the Section 7 and the help page of the function (`?msaPrettyPrint`).

The `msaPrettyPrint()` function is particularly useful for pretty-printing multiple sequence alignments in Sweave [6] or knitr [15] documents. More details are provided in Section 7. Here, we restrict to a teasing example:

```
msaPrettyPrint(myFirstAlignment, y=c(164, 213), output="asis",
               showNames="none", showLogo="none", askForOverwrite=FALSE)
```

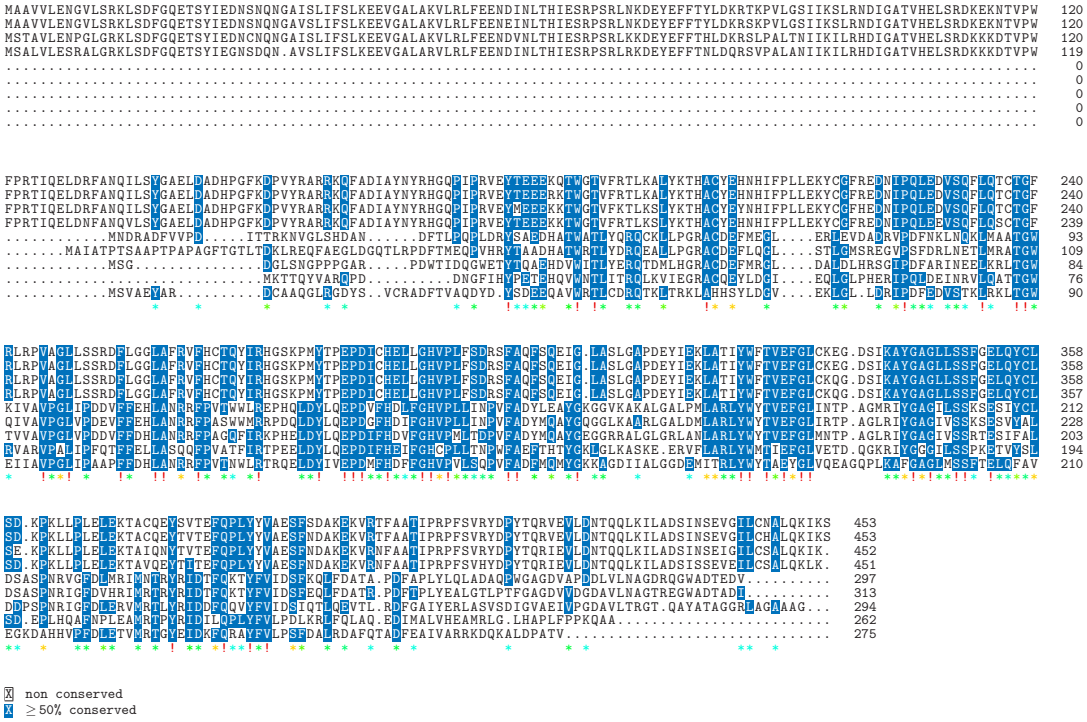


Figure 1: The PDF file `myfirstAlignment.pdf` created with `msaPrettyPrint()`.

4 Functions for Multiple Sequence Alignment in More Detail

The example in Section 3 above simply called the function `msa()` without any additional arguments. We mentioned already that, in this case, ClustalW is called with default parameters. We can also explicitly request ClustalW or one of the two other algorithms ClustalOmega or MUSCLE:

```
myClustalWAlignment <- msa(mySequences, "ClustalW")

## use default substitution matrix

myClustalWAlignment

## CLUSTAL 2.1
##
## Call:
##    msa(mySequences, "ClustalW")
##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln                                        names
## [1] MAAVVLENGVLSRKLSDF...SINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] MAAVVLENGVLSRKLSDF...SINSEVGILCHALQKIKS PH4H_Mus_musculus
## [3] MSTAVLENPGLGRKLSDF...SINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [4] MSALVLESRALGRKLSDF...SISSEVEILCSALQKLK- PH4H_Bos_taurus
## [5] ------------------...GWADTEDV---------- PH4H_Chromobacter...
## [6] ------------------...GWADTADI---------- PH4H_Ralstonia_so...
## [7] ------------------...AYATAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8] ------------------...------------------ PH4H_Pseudomonas_...
## [9] ------------------...------------------ PH4H_Rhizobium_loti
## Con ------------------...???????IL??A???--- Consensus

myClustalOmegaAlignment <- msa(mySequences, "ClustalOmega")
```

```
## using Gonnet

myClustalOmegaAlignment

## ClustalOmega 1.2.0
##
## Call:
##    msa(mySequences, "ClustalOmega")
##
## MsaAAMultipleAlignment with 9 rows and 467 columns
##     aln                                        names
## [1] MSALVLESRALGRKLSDF...SISSEVEILCSALQKLK- PH4H_Bos_taurus
## [2] MSTAVLENPGLGRKLSDF...SINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [3] MAAVVLENGVLSRKLSDF...SINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [4] MAAVVLENGVLSRKLSDF...SINSEVGILCHALQKIKS PH4H_Mus_musculus
## [5] ------------------...------------------ PH4H_Pseudomonas_...
## [6] ------------------...------------------ PH4H_Rhizobium_loti
## [7] ------------------...LAGAAAG----------- PH4H_Caulobacter_...
## [8] ------------------...V----------------- PH4H_Chromobacter...
## [9] ------------------...I----------------- PH4H_Ralstonia_so...
## Con ------------------...???????----------- Consensus

myMuscleAlignment <- msa(mySequences, "Muscle")
myMuscleAlignment

## MUSCLE 3.8.31
##
## Call:
##    msa(mySequences, "Muscle")
##
## MsaAAMultipleAlignment with 9 rows and 460 columns
##     aln                                        names
## [1] MAAVVLENGVLSRKLSDF...SINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] MAAVVLENGVLSRKLSDF...SINSEVGILCHALQKIKS PH4H_Mus_musculus
## [3] MSTAVLENPGLGRKLSDF...SINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [4] MSALVLESRALGRKLSDF...SISSEVEILCSALQKLK- PH4H_Bos_taurus
## [5] ------------------...------------------ PH4H_Pseudomonas_...
## [6] ------------------...------------------ PH4H_Rhizobium_loti
## [7] ------------------...AYATAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8] -----------MNDRADF...QGWADTEDV--------- PH4H_Chromobacter...
## [9] MAIATPTSAAPTPAPAGF...EGWADTADI--------- PH4H_Ralstonia_so...
## Con M???????????????DF...????????L??A???--- Consensus
```

Please note that the call `msa(mySequences, "ClustalW", ...)` is just a shortcut for the call `msaClustalW(mySequences, ...)`, analogously for `msaClustalOmega()` and `msaMuscle()`.

In other words, `msa()` is nothing else but a wrapper function that provides a unified interface to the three functions `msaClustalW()`, `msaClustalOmega()`, and `msaMuscle()`.

All three functions `msaClustalW()`, `msaClustalOmega()`, and `msaMuscle()` have the same parameters: The input sequences are passed as argument `inputSeqs`, and all functions have the following arguments: `cluster`, `gapOpening`, `gapExtension`, `maxiters`, `substitutionMatrix`, `order`, `type`, and `verbose`. The ways these parameters are interpreted, are largely analogous, although there are some differences, also in terms of default values. See the subsections below and the man page of the three functions for more details. All of the three functions `msaClustalW()`, `msaClustalOmega()`, and `msaMuscle()`, however, are not restricted to the parameters mentioned above. All three have a '`...`' argument through which several other algorithm-specific parameters can be passed on to the underlying library. The following subsections provide an overview of which parameters are supported by each of the three algorithms.

4.1 ClustalW-Specific Parameters

The original implementation of ClustalW offers a lot of parameters for customizing the way a multiple sequence alignment is computed. Through the '`...`' argument, `msaClustalW()` provides an interface to make use of most these parameters (see the documentation of ClustalW[2] for a comprehensive overview). Currently, the following restrictions and caveats apply:

- The parameters `infile`, `clustering`, `gapOpen`, `gapExt`, `numiters`, `matrix`, and `outorder` have been renamed to the standardized argument names `inputSeqs`, `cluster`, `gapOpening`, `gapExtension`, `maxiters`, `substitutionMatrix`, and `order` in order to provide a consistent interface for all three multiple sequence alignment algorithms.
- Boolean flags must be passed as logical values, e.g. `verbose=TRUE`.
- The parameter `quiet` has been replaced by `verbose` (with the exact opposite meaning).
- The following parameters are (currently) not supported: `bootstrap`, `check`, `fullhelp`, `interactive`, `maxseqlen`, `options`, and `tree`.
- For the parameter `output`, only the choice `"clustal"` is available.

4.2 ClustalOmega-Specific Parameters

In the same way as ClustalW, the original implementation of ClustalOmega also offers a lot of parameters for customizing the way a multiple sequence alignment is computed. Through the '`...`' argument, `msaClustalOmega()` provides an interface to make use of most these parameters (see the documentation of ClustalOmega[3] for a comprehensive overview). Currently, the following restrictions and caveats apply:

- The parameters `infile`, `cluster-size`, `iterations`, and `output-order` have been renamed to the argument names `inputSeqs`, `cluster`, `maxiters`, and `order` in order to provide a consistent interface for all three multiple sequence alignment algorithms.

[2] `http://www.clustal.org/download/clustalw_help.txt`
[3] `http://www.clustal.org/omega/README`

- ClustalOmega does not allow for setting custom gap penalties. Therefore, setting the parameters `gapOpening` and `gapExtension` currently has no effect and will lead to a warning. These arguments are only defined for future extensions and consistency with the other algorithms available in `msa`.
- ClustalOmega only allows for choosing substitution matrices from a pre-defined set of names, namely `"BLOSUM30"`, `"BLOSUM40"`, `"BLOSUM50"`, `"BLOSUM65"`, `"BLOSUM80"`, and `"Gonnet"`. This is a new feature — the original ClustalOmega implementation does not allow for using any custom substitution matrix. However, since these are all amino acid substitution matrices, ClustalOmega is still hardly useful for multiple alignments of nucleotide sequences.
- Boolean flags must be passed as logical values, e.g. `verbose=TRUE`.
- The following parameters are (currently) not supported: `maxSeqLength` and `help`.
- For the parameter `outFmt`, only the choice `"clustal"` is available.

4.3 MUSCLE-Specific Parameters

Finally, also MUSCLE offers a lot of parameters for customizing the way a multiple sequence alignment is computed. Through the '`...`' argument, `msaMuscle()` provides an interface to make use of most these parameters (see the documentation of MUSCLE[4] for a comprehensive overview). Currently, the following restrictions and caveats apply:

- The parameters `in`, `gapOpen`, `gapExtend`, `matrix`, and `seqtype` have been renamed to `inputSeqs`, `gapOpening`, `gapExtension`, `substitutionMatrix` and `type` in order to provide a consistent interface for all three multiple sequence alignment algorithms.
- Boolean flags must be passed as logical values, e.g. `verbose=TRUE`.
- The parameter `quiet` has been replaced by `verbose` (with the exact opposite meaning).
- The following parameters are currently not supported: `clw`, `clwstrict`, `fastaout`, `group`, `html`, `in1`, `in2`, `log`, `loga`, `msaout`, `msf`, `out`, `phyi`, `phyiout`, `phys`, `physout`, `refine`, `refinew`, `scorefile`, `spscore`, `stable`, `termgaps4`, `termgapsfull`, `termgapshalf`, `termgapshalflonger`, `tree1`, `tree2`, `usetree`, `weight1`, and `weight2`.

5 Printing Multiple Sequence Alignments

As already shown above, multiple sequence alignments can be shown in plain text format on the `R` console using the `print()` function (which is implicitly called if just the object name is entered on the `R` console). This function allows for multiple customizations, such as, enabling/disabling to display a consensus sequence, printing the entire alignment or only a subset, enabling/disabling to display sequence names, and adjusting the width allocated for sequence names. For more information, the reader is referred to the help page of the print function:

[4] `http://www.drive5.com/muscle/muscle.html`

```
help("print,MsaDNAMultipleAlignment-method")
```

We only provide some examples here:

```
print(myFirstAlignment)

## CLUSTAL 2.1
##
## Call:
##    msa(mySequences)
##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln                                        names
## [1] MAAVVLENGVLSRKLSDF...SINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] MAAVVLENGVLSRKLSDF...SINSEVGILCHALQKIKS PH4H_Mus_musculus
## [3] MSTAVLENPGLGRKLSDF...SINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [4] MSALVLESRALGRKLSDF...SISSEVEILCSALQKLK- PH4H_Bos_taurus
## [5] ------------------...GWADTEDV---------- PH4H_Chromobacter...
## [6] ------------------...GWADTADI---------- PH4H_Ralstonia_so...
## [7] ------------------...AYATAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8] ------------------...------------------ PH4H_Pseudomonas_...
## [9] ------------------...------------------ PH4H_Rhizobium_loti
## Con ------------------...???????IL??A???--- Consensus

print(myFirstAlignment, show="complete")

##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln (1..39)                                names
## [1] MAAVVLENGVLSRKLSDFGQETSYIEDNSNQNGAISLIF PH4H_Rattus_norve...
## [2] MAAVVLENGVLSRKLSDFGQETSYIEDNSNQNGAVSLIF PH4H_Mus_musculus
## [3] MSTAVLENPGLGRKLSDFGQETSYIEDNCNQNGAISLIF PH4H_Homo_sapiens
## [4] MSALVLESRALGRKLSDFGQETSYIEGNSDQN-AVSLIF PH4H_Bos_taurus
## [5] ---------------------------------------- PH4H_Chromobacter...
## [6] ---------------------------------------- PH4H_Ralstonia_so...
## [7] ---------------------------------------- PH4H_Caulobacter_...
## [8] ---------------------------------------- PH4H_Pseudomonas_...
## [9] ---------------------------------------- PH4H_Rhizobium_loti
## Con ---------------------------------------- Consensus
##
##     aln (40..78)                               names
## [1] SLKEEVGALAKVLRLFEENDINLTHIESRPSRLNKDEYE PH4H_Rattus_norve...
## [2] SLKEEVGALAKVLRLFEENEINLTHIESRPSRLNKDEYE PH4H_Mus_musculus
## [3] SLKEEVGALAKVLRLFEENDVNLTHIESRPSRLKKDEYE PH4H_Homo_sapiens
## [4] SLKEEVGALARVLRLFEENDINLTHIESRPSRLRKDEYE PH4H_Bos_taurus
```

```
## [5] ---------------------------------------- PH4H_Chromobacter...
## [6] ---------------------------------------- PH4H_Ralstonia_so...
## [7] ---------------------------------------- PH4H_Caulobacter_...
## [8] ---------------------------------------- PH4H_Pseudomonas_...
## [9] ---------------------------------------- PH4H_Rhizobium_loti
## Con ---------------------------------------- Consensus
##
##     aln (79..117)                            names
## [1] FFTYLDKRTKPVLGSIIKSLRNDIGATVHELSRDKEKNT PH4H_Rattus_norve...
## [2] FFTYLDKRSKPVLGSIIKSLRNDIGATVHELSRDKEKNT PH4H_Mus_musculus
## [3] FFTHLDKRSLPALTNIIKILRHDIGATVHELSRDKKKDT PH4H_Homo_sapiens
## [4] FFTNLDQRSVPALANIIKILRHDIGATVHELSRDKKKDT PH4H_Bos_taurus
## [5] ---------------------------------------- PH4H_Chromobacter...
## [6] ---------------------------------------- PH4H_Ralstonia_so...
## [7] ---------------------------------------- PH4H_Caulobacter_...
## [8] ---------------------------------------- PH4H_Pseudomonas_...
## [9] ---------------------------------------- PH4H_Rhizobium_loti
## Con ---------------------------------------- Consensus
##
##     aln (118..156)                           names
## [1] VPWFPRTIQELDRFANQILSYGAELDADHPGFKDPVYRA PH4H_Rattus_norve...
## [2] VPWFPRTIQELDRFANQILSYGAELDADHPGFKDPVYRA PH4H_Mus_musculus
## [3] VPWFPRTIQELDRFANQILSYGAELDADHPGFKDPVYRA PH4H_Homo_sapiens
## [4] VPWFPRTIQELDNFANQVLSYGAELDADHPGFKDPVYRA PH4H_Bos_taurus
## [5] ---------------MNDRADFVVPD-----ITTRKNVG PH4H_Chromobacter...
## [6] ----------MAIATPTSAAPTPAPAGFTGTLTDKLREQ PH4H_Ralstonia_so...
## [7] ---------------MSG---------------DGLSNG PH4H_Caulobacter_...
## [8] ---------------------------------MKTTQY PH4H_Pseudomonas_...
## [9] ---------------MSVAEYAR----------DCAAQG PH4H_Rhizobium_loti
## Con ----------??????????Y????D???????D????? Consensus
##
##     aln (157..195)                           names
## [1] RRKQFADIAYNYRHGQPIPRVEYTEEEKQTWGTVFRTLK PH4H_Rattus_norve...
## [2] RRKQFADIAYNYRHGQPIPRVEYTEEERKTWGTVFRTLK PH4H_Mus_musculus
## [3] RRKQFADIAYNYRHGQPIPRVEYMEEEKKTWGTVFKTLK PH4H_Homo_sapiens
## [4] RRKQFADIAYNYRHGQPIPRVEYTEEEKKTWGTVFRTLK PH4H_Bos_taurus
## [5] LSHDAN------DFTLPQPLDRYSAEDHATWATLYQRQC PH4H_Chromobacter...
## [6] FAEGLDGQTLRPDFTMEQPVHRYTAADHATWRTLYDRQE PH4H_Ralstonia_so...
## [7] PPPGAR-----PDWTIDQGWETYTQAEHDVWITLYERQT PH4H_Caulobacter_...
## [8] VARQPD----------DNGFIHYPETEHQVWNTLITRQL PH4H_Pseudomonas_...
## [9] LRGDYS--VCRADFTVAQDYD-YSDEEQAVWRTLCDRQT PH4H_Rhizobium_loti
## Con ?R?Q???????????P?P???YTEEE??TW?TL??RQ? Consensus
##
##     aln (196..234)                           names
## [1] ALYKTHACYEHNHIFPLLEKYCGFREDNIPQLEDVSQFL PH4H_Rattus_norve...
## [2] ALYKTHACYEHNHIFPLLEKYCGFREDNIPQLEDVSQFL PH4H_Mus_musculus
```

```
## [3] SLYKTHACYEYNHIFPLLEKYCGFHEDNIPQLEDVSQFL PH4H_Homo_sapiens
## [4] SLYKTHACYEHNHIFPLLEKYCGFREDNIPQLEEVSQFL PH4H_Bos_taurus
## [5] KLLPGRACDEFMEGL----ERLEVDADRVPDFNKLNQKL PH4H_Chromobacter...
## [6] ALLPGRACDEFLQGL----STLGMSREGVPSFDRLNETL PH4H_Ralstonia_so...
## [7] DMLHGRACDEFMRGL----DALDLHRSGIPDFARINEEL PH4H_Caulobacter_...
## [8] KVIEGRACQEYLDGI----EQLGLPHERIPQLDEINRVL PH4H_Pseudomonas_...
## [9] KLTRKLAHHSYLDGV----EKLGL-LDRIPDFEDVSTKL PH4H_Rhizobium_loti
## Con ?L????AC?E???G?----??LG???D?IPQLE?VSQ?L Consensus
##
##     aln (235..273)                          names
## [1] QTCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Rattus_norve...
## [2] QTCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Mus_musculus
## [3] QTCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Homo_sapiens
## [4] QSCTGFRLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGS PH4H_Bos_taurus
## [5] MAATGWKIVAVPGLIPDDVFFEHLANRRFPVTWWLREPH PH4H_Chromobacter...
## [6] MRATGWQIVAVPGLVPDEVFFEHLANRRFPASWWMRRPD PH4H_Ralstonia_so...
## [7] KRLTGWTVVAVPGLVPDDVFFDHLANRRFPAGQFIRKPH PH4H_Caulobacter_...
## [8] QATTGWRVARVPALIPFQTFFELLASQQFPVATFIRTPE PH4H_Pseudomonas_...
## [9] RKLTGWEIIAVPGLIPAAPFFDHLANRRFPVTNWLRTRQ PH4H_Rhizobium_loti
## Con Q??TGWR???VPGL?P???FF??LA?R?FP?TQ?IR??? Consensus
##
##     aln (274..312)                          names
## [1] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Rattus_norve...
## [2] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Mus_musculus
## [3] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Homo_sapiens
## [4] KPMYTPEPDICHELLGHVPLFSDRSFAQFSQEIG-LASL PH4H_Bos_taurus
## [5] QLDYLQEPDVFHDLFGHVPLLINPVFADYLEAYGKGGVK PH4H_Chromobacter...
## [6] QLDYLQEPDGFHDIFGHVPLLINPVFADYMQAYGQGGLK PH4H_Ralstonia_so...
## [7] ELDYLQEPDIFHDVFGHVPMLTDPVFADYMQAYGEGGRR PH4H_Caulobacter_...
## [8] ELDYLQEPDIFHEIFGHCPLLTNPWFAEFTHTYGKLGLK PH4H_Pseudomonas_...
## [9] ELDYIVEPDMFHDFFGHVPVLSQPVFADFMQMYGKKAGD PH4H_Rhizobium_loti
## Con ?LDY??EPDIFHELFGHVPLLSDP?FA?F?Q?YG?LA?? Consensus
##
##     aln (313..351)                          names
## [1] GAPDEYIEKLATIYWFTVEFGLCKEG-DSIKAYGAGLLS PH4H_Rattus_norve...
## [2] GAPDEYIEKLATIYWFTVEFGLCKEG-DSIKAYGAGLLS PH4H_Mus_musculus
## [3] GAPDEYIEKLATIYWFTVEFGLCKQG-DSIKAYGAGLLS PH4H_Homo_sapiens
## [4] GAPDEYIEKLATIYWFTVEFGLCKQG-DSIKAYGAGLLS PH4H_Bos_taurus
## [5] AKALGALPMLARLYWYTVEFGLINTP-AGMRIYGAGILS PH4H_Chromobacter...
## [6] AARLGALDMLARLYWYTVEFGLIRTP-AGLRIYGAGIVS PH4H_Ralstonia_so...
## [7] ALGLGRLANLARLYWYTVEFGLMNTP-AGLRIYGAGIVS PH4H_Caulobacter_...
## [8] ASKE-ERVFLARLYWMTIEFGLVETD-QGKRIYGGGILS PH4H_Pseudomonas_...
## [9] IIALGGDEMITRLYWYTAEYGLVQEAGQPLKAFGAGLMS PH4H_Rhizobium_loti
## Con ?A?????E?LARLYW?TVEFGL????-???KAYGAGLLS Consensus
##
##     aln (352..390)                          names
```

```
## [1] SFGELQYCLSD-KPKLLPLELEKTACQEYSVTEFQPLYY PH4H_Rattus_norve...
## [2] SFGELQYCLSD-KPKLLPLELEKTACQEYTVTEFQPLYY PH4H_Mus_musculus
## [3] SFGELQYCLSE-KPKLLPLELEKTAIQNYTVTEFQPLYY PH4H_Homo_sapiens
## [4] SFGELQYCLSD-KPKLLPLELEKTAVQEYTITEFQPLYY PH4H_Bos_taurus
## [5] SKSESIYCLDSASPNRVGFDLMRIMNTRYRIDTFQKTYF PH4H_Chromobacter...
## [6] SKSESVYALDSASPNRIGFDVHRIMRTRYRIDTFQKTYF PH4H_Ralstonia_so...
## [7] SRTESIFALDDPSPNRIGFDLERVMRTLYRIDDFQQVYF PH4H_Caulobacter_...
## [8] SPKETVYSLSD-EPLHQAFNPLEAMRTPYRIDILQPLYF PH4H_Pseudomonas_...
## [9] SFTELQFAVEGKDAHHVPFDLETVMRTGYEIDKFQRAYF PH4H_Rhizobium_loti
## Con SF?ELQYCLSD-?P???PF?LE??M?T?Y?ID?FQPLYF Consensus
##
##     aln (391..429)                          names
## [1] VAESFSDAKEKVRTFAATIPRPFSVRYDPYTQRVEVLDN PH4H_Rattus_norve...
## [2] VAESFNDAKEKVRTFAATIPRPFSVRYDPYTQRVEVLDN PH4H_Mus_musculus
## [3] VAESFNDAKEKVRNFAATIPRPFSVRYDPYTQRIEVLDN PH4H_Homo_sapiens
## [4] VAESFNDAKEKVRNFAATIPRPFSVHYDPYTQRIEVLDN PH4H_Bos_taurus
## [5] VIDSFKQLFDATA-PDFAPLYLQLADAQPWGAGDVAPDD PH4H_Chromobacter...
## [6] VIDSFEQLFDATR-PDFTPLYEALGTLPTFGAGDVVDGD PH4H_Ralstonia_so...
## [7] VIDSIQTLQEVTL-RDFGAIYERLASVSDIGVAEIVPGD PH4H_Caulobacter_...
## [8] VLPDLKRLFQLAQ-EDIMALVHEAMRLG-LHAPLFPPKQ PH4H_Pseudomonas_...
## [9] VLPSFDALRDAFQTADFEAIVARRKDQKALDPATV---- PH4H_Rhizobium_loti
## Con V??SF??L?E??R??D?T??????????P??????V?D? Consensus
##
##     aln (430..456)              names
## [1] TQQLKILADSINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] TQQLKILADSINSEVGILCHALQKIKS PH4H_Mus_musculus
## [3] TQQLKILADSINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [4] TQQLKILADSISSEVEILCSALQKLK- PH4H_Bos_taurus
## [5] LVLNAGDRQGWADTEDV---------- PH4H_Chromobacter...
## [6] AVLNAGTREGWADTADI---------- PH4H_Ralstonia_so...
## [7] AVLTRGT-QAYATAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8] AA------------------------- PH4H_Pseudomonas_...
## [9] --------------------------- PH4H_Rhizobium_loti
## Con ?????????????????IL??A???--- Consensus
```

```r
print(myFirstAlignment, showConsensus=FALSE, halfNrow=3)
```

```
## CLUSTAL 2.1
##
## Call:
##    msa(mySequences)
##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln                                     names
## [1] MAAVVLENGVLSRKLSDF...SINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] MAAVVLENGVLSRKLSDF...SINSEVGILCHALQKIKS PH4H_Mus_musculus
```

```
## [3] MSTAVLENPGLGRKLSDF...SINSEIGILCSALQKIK- PH4H_Homo_sapiens
## ... ...
## [7] ------------------...AYATAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8] ------------------...------------------ PH4H_Pseudomonas_...
## [9] ------------------...------------------ PH4H_Rhizobium_loti

print(myFirstAlignment, showNames=FALSE, show="complete")

##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln (1..60)
## [1] MAAVVLENGVLSRKLSDFGQETSYIEDNSNQNGAISLIFSLKEEVGALAKVLRLFEENDI
## [2] MAAVVLENGVLSRKLSDFGQETSYIEDNSNQNGAVSLIFSLKEEVGALAKVLRLFEENEI
## [3] MSTAVLENPGLGRKLSDFGQETSYIEDNCNQNGAISLIFSLKEEVGALAKVLRLFEENDV
## [4] MSALVLESRALGRKLSDFGQETSYIEGNSDQN-AVSLIFSLKEEVGALARVLRLFEENDI
## [5] ------------------------------------------------------------
## [6] ------------------------------------------------------------
## [7] ------------------------------------------------------------
## [8] ------------------------------------------------------------
## [9] ------------------------------------------------------------
## Con ------------------------------------------------------------
##
##     aln (61..120)
## [1] NLTHIESRPSRLNKDEYEFFTYLDKRTKPVLGSIIKSLRNDIGATVHELSRDKEKNTVPW
## [2] NLTHIESRPSRLNKDEYEFFTYLDKRSKPVLGSIIKSLRNDIGATVHELSRDKEKNTVPW
## [3] NLTHIESRPSRLKKDEYEFFTHLDKRSLPALTNIIKILRHDIGATVHELSRDKKKDTVPW
## [4] NLTHIESRPSRLRKDEYEFFTNLDQRSVPALANIIKILRHDIGATVHELSRDKKKDTVPW
## [5] ------------------------------------------------------------
## [6] ------------------------------------------------------------
## [7] ------------------------------------------------------------
## [8] ------------------------------------------------------------
## [9] ------------------------------------------------------------
## Con ------------------------------------------------------------
##
##     aln (121..180)
## [1] FPRTIQELDRFANQILSYGAELDADHPGFKDPVYRARRKQFADIAYNYRHGQPIPRVEYT
## [2] FPRTIQELDRFANQILSYGAELDADHPGFKDPVYRARRKQFADIAYNYRHGQPIPRVEYT
## [3] FPRTIQELDRFANQILSYGAELDADHPGFKDPVYRARRKQFADIAYNYRHGQPIPRVEYM
## [4] FPRTIQELDNFANQVLSYGAELDADHPGFKDPVYRARRKQFADIAYNYRHGQPIPRVEYT
## [5] ------------MNDRADFVVPD-----ITTRKNVGLSHDAN------DFTLPQPLDRYS
## [6] -------MAIATPTSAAPTPAPAGFTGTLTDKLREQFAEGLDGQTLRPDFTMEQPVHRYT
## [7] ------------MSG---------------DGLSNGPPPGAR-----PDWTIDQGWETYT
## [8] ------------------------------MKTTQYVARQPD----------DNGFIHYP
## [9] ------------MSVAEYAR----------DCAAQGLRGDYS--VCRADFTVAQDYD-YS
## Con -------?????????Y????D???????D??????R?Q???????????P?P???YT
##
```

```
##     aln (181..240)
## [1] EEEKQTWGTVFRTLKALYKTHACYEHNHIFPLLEKYCGFREDNIPQLEDVSQFLQTCTGF
## [2] EEERKTWGTVFRTLKALYKTHACYEHNHIFPLLEKYCGFREDNIPQLEDVSQFLQTCTGF
## [3] EEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLLEKYCGFHEDNIPQLEDVSQFLQTCTGF
## [4] EEEKKTWGTVFRTLKSLYKTHACYEHNHIFPLLEKYCGFREDNIPQLEEVSQFLQSCTGF
## [5] AEDHATWATLYQRQCKLLPGRACDEFMEGL----ERLEVDADRVPDFNKLNQKLMAATGW
## [6] AADHATWRTLYDRQEALLPGRACDEFLQGL----STLGMSREGVPSFDRLNETLMRATGW
## [7] QAEHDVWITLYERQTDMLHGRACDEFMRGL----DALDLHRSGIPDFARINEELKRLTGW
## [8] ETEHQVWNTLITRQLKVIEGRACQEYLDGI----EQLGLPHERIPQLDEINRVLQATTGW
## [9] DEEQAVWRTLCDRQTKLTRKLAHHSYLDGV----EKLGL-LDRIPDFEDVSTKLRKLTGW
## Con EEE??TW?TL??RQ??L????AC?E???G?----??LG???D?IPQLE?VSQ?LQ??TGW
##
##     aln (241..300)
## [1] RLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGSKPMYTPEPDICHELLGHVPLFSDRSFA
## [2] RLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGSKPMYTPEPDICHELLGHVPLFSDRSFA
## [3] RLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGSKPMYTPEPDICHELLGHVPLFSDRSFA
## [4] RLRPVAGLLSSRDFLGGLAFRVFHCTQYIRHGSKPMYTPEPDICHELLGHVPLFSDRSFA
## [5] KIVAVPGLIPDDVFFEHLANRRFPVTWWLREPHQLDYLQEPDVFHDLFGHVPLLINPVFA
## [6] QIVAVPGLVPDEVFFEHLANRRFPASWWMRRPDQLDYLQEPDGFHDIFGHVPLLINPVFA
## [7] TVVAVPGLVPDDVFFDHLANRRFPAGQFIRKPHELDYLQEPDIFHDVFGHVPMLTDPVFA
## [8] RVARVPALIPFQTFFELLASQQFPVATFIRTPEELDYLQEPDIFHEIFGHCPLLTNPWFA
## [9] EIIAVPGLIPAAPFFDHLANRRFPVTNWLRTRQELDYIVEPDMFHDFFGHVPVLSQPVFA
## Con R???VPGL?P???FF??LA?R?FP?TQ?IR????LDY??EPDIFHELFGHVPLLSDP?FA
##
##     aln (301..360)
## [1] QFSQEIG-LASLGAPDEYIEKLATIYWFTVEFGLCKEG-DSIKAYGAGLLSSFGELQYCL
## [2] QFSQEIG-LASLGAPDEYIEKLATIYWFTVEFGLCKEG-DSIKAYGAGLLSSFGELQYCL
## [3] QFSQEIG-LASLGAPDEYIEKLATIYWFTVEFGLCKQG-DSIKAYGAGLLSSFGELQYCL
## [4] QFSQEIG-LASLGAPDEYIEKLATIYWFTVEFGLCKQG-DSIKAYGAGLLSSFGELQYCL
## [5] DYLEAYGKGGVKAKALGALPMLARLYWYTVEFGLINTP-AGMRIYGAGILSSKSESIYCL
## [6] DYMQAYGQGGLKAARLGALDMLARLYWYTVEFGLIRTP-AGLRIYGAGIVSSKSESVYAL
## [7] DYMQAYGEGGRRALGLGRLANLARLYWYTVEFGLMNTP-AGLRIYGAGIVSSRTESIFAL
## [8] EFTHTYGKLGLKASKE-ERVFLARLYWMTIEFGLVETD-QGKRIYGGGILSSPKETVYSL
## [9] DFMQMYGKKAGDIIALGGDEMITRLYWYTAEYGLVQEAGQPLKAFGAGLMSSFTELQFAV
## Con ?F?Q?YG?LA???A?????E?LARLYW?TVEFGL????-???KAYGAGLLSSF?ELQYCL
##
##     aln (361..420)
## [1] SD-KPKLLPLELEKTACQEYSVTEFQPLYYVAESFSDAKEKVRTFAATIPRPFSVRYDPY
## [2] SD-KPKLLPLELEKTACQEYTVTEFQPLYYVAESFNDAKEKVRTFAATIPRPFSVRYDPY
## [3] SE-KPKLLPLELEKTAIQNYTVTEFQPLYYVAESFNDAKEKVRNFAATIPRPFSVRYDPY
## [4] SD-KPKLLPLELEKTAVQEYTITEFQPLYYVAESFNDAKEKVRNFAATIPRPFSVHYDPY
## [5] DSASPNRVGFDLMRIMNTRYRIDTFQKTYFVIDSFKQLFDATA-PDFAPLYLQLADAQPW
## [6] DSASPNRIGFDVHRIMRTRYRIDTFQKTYFVIDSFEQLFDATR-PDFTPLYEALGTLPTF
## [7] DDPSPNRIGFDLERVMRTLYRIDDFQQVYFVIDSIQTLQEVTL-RDFGAIYERLASVSDI
## [8] SD-EPLHQAFNPLEAMRTPYRIDILQPLYFVLPDLKRLFQLAQ-EDIMALVHEAMRLG-L
## [9] EGKDAHHVPFDLETVMRTGYEIDKFQRAYFVLPSFDALRDAFQTADFEAIVARRKDQKAL
```

```
## Con SD-?P???PF?LE??M?T?Y?ID?FQPLYFV??SF??L?E??R??D?T??????????P?
##
##     aln (421..456)
## [1] TQRVEVLDNTQQLKILADSINSEVGILCNALQKIKS
## [2] TQRVEVLDNTQQLKILADSINSEVGILCHALQKIKS
## [3] TQRIEVLDNTQQLKILADSINSEIGILCSALQKIK-
## [4] TQRIEVLDNTQQLKILADSISSEVEILCSALQKLK-
## [5] GAGDVAPDDLVLNAGDRQGWADTEDV----------
## [6] GAGDVVDGDAVLNAGTREGWADTADI----------
## [7] GVAEIVPGDAVLTRGT-QAYATAGGRLAGAAAG---
## [8] HAPLFPPKQAA-------------------------
## [9] DPATV-------------------------------
## Con ?????V?D?????????????????IL??A???---
```

6 Processing Multiple Alignments

6.1 Methods Inherited From `Biostrings`

The classes defined by the `msa` package for storing multiple alignment results have been derived from the corresponding classes defined by the `Biostrings` package. Therefore, all methods for processing multiple alignments are available and work without any practical limitation. In this section, we highlight some of these.

The classes used for storing multiple alignments allow for defining masks on sequences and sequence positions via their row and column mask slots. They can be set by `rowmask()` and `colmask()` functions which serve both as setter and getter functions. To set row or column masks, an `IRanges` object must be supplied:

```
myMaskedAlignment <- myFirstAlignment
colM <- IRanges(start=1, end=100)
colmask(myMaskedAlignment) <- colM
myMaskedAlignment
```

```
## CLUSTAL 2.1
##
## Call:
##    msa(mySequences)
##
## MsaAAMultipleAlignment with 9 rows and 456 columns
##     aln                                      names
## [1] ##################...SINSEVGILCNALQKIKS PH4H_Rattus_norve...
## [2] ##################...SINSEVGILCHALQKIKS PH4H_Mus_musculus
## [3] ##################...SINSEIGILCSALQKIK- PH4H_Homo_sapiens
## [4] ##################...SISSEVEILCSALQKLK- PH4H_Bos_taurus
## [5] ##################...GWADTEDV---------- PH4H_Chromobacter...
```

```
## [6] ##################...GWADTADI---------- PH4H_Ralstonia_so...
## [7] ##################...AYATAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8] ##################...------------------ PH4H_Pseudomonas_...
## [9] ##################...------------------ PH4H_Rhizobium_loti
## Con ##################...???????IL??A???--- Consensus
```

The `unmasked()` allows for removing these masks, thereby casting the multiple alignment to a set of aligned `Biostrings` sequences (class `AAStringSet`, `DNAStringSet`, or `RNAStringSet`):

```
unmasked(myMaskedAlignment)

##   A AAStringSet instance of length 9
##     width seq                                  names
## [1]   456 MAAVVLENGVLSRKLS...SEVGILCNALQKIKS PH4H_Rattus_norve...
## [2]   456 MAAVVLENGVLSRKLS...SEVGILCHALQKIKS PH4H_Mus_musculus
## [3]   456 MSTAVLENPGLGRKLS...SEIGILCSALQKIK- PH4H_Homo_sapiens
## [4]   456 MSALVLESRALGRKLS...SEVEILCSALQKLK- PH4H_Bos_taurus
## [5]   456 ----------------...DTEDV---------- PH4H_Chromobacter...
## [6]   456 ----------------...DTADI---------- PH4H_Ralstonia_so...
## [7]   456 ----------------...TAGGRLAGAAAG--- PH4H_Caulobacter_...
## [8]   456 ----------------...--------------- PH4H_Pseudomonas_...
## [9]   456 ----------------...--------------- PH4H_Rhizobium_loti
```

Consensus matrices can be computed conveniently as follows:

```
conMat <- consensusMatrix(myFirstAlignment)
dim(conMat)

## [1]  21 456

conMat[, 101:110]

##   [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10]
## -    5    5    5    5    5    5    5    5    5     5
## A    0    0    0    4    0    0    0    0    0     0
## C    0    0    0    0    0    0    0    0    0     0
## D    4    0    0    0    0    0    0    0    0     0
## E    0    0    0    0    0    0    0    4    0     0
## F    0    0    0    0    0    0    0    0    0     0
## G    0    0    4    0    0    0    0    0    0     0
## H    0    0    0    0    0    0    4    0    0     0
## I    0    4    0    0    0    0    0    0    0     0
## K    0    0    0    0    0    0    0    0    0     0
## L    0    0    0    0    0    0    0    0    4     0
```

```
## M    0    0    0    0    0    0    0    0    0    0
## N    0    0    0    0    0    0    0    0    0    0
## P    0    0    0    0    0    0    0    0    0    0
## Q    0    0    0    0    0    0    0    0    0    0
## R    0    0    0    0    0    0    0    0    0    0
## S    0    0    0    0    0    0    0    0    0    4
## T    0    0    0    0    4    0    0    0    0    0
## V    0    0    0    0    0    4    0    0    0    0
## W    0    0    0    0    0    0    0    0    0    0
## Y    0    0    0    0    0    0    0    0    0    0
```

If called on a masked alignment, `consensusMatrix()` only uses those sequences/rows that are not masked. If there are masked columns, the matrix contains `NA`'s in those columns:

```
conMat <- consensusMatrix(myMaskedAlignment)
conMat[, 95:104]
```

```
##   [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10]
## -   NA   NA   NA   NA   NA   NA    5    5    5     5
## A   NA   NA   NA   NA   NA   NA    0    0    0     4
## C   NA   NA   NA   NA   NA   NA    0    0    0     0
## D   NA   NA   NA   NA   NA   NA    4    0    0     0
## E   NA   NA   NA   NA   NA   NA    0    0    0     0
## F   NA   NA   NA   NA   NA   NA    0    0    0     0
## G   NA   NA   NA   NA   NA   NA    0    0    4     0
## H   NA   NA   NA   NA   NA   NA    0    0    0     0
## I   NA   NA   NA   NA   NA   NA    0    4    0     0
## K   NA   NA   NA   NA   NA   NA    0    0    0     0
## L   NA   NA   NA   NA   NA   NA    0    0    0     0
## M   NA   NA   NA   NA   NA   NA    0    0    0     0
## N   NA   NA   NA   NA   NA   NA    0    0    0     0
## P   NA   NA   NA   NA   NA   NA    0    0    0     0
## Q   NA   NA   NA   NA   NA   NA    0    0    0     0
## R   NA   NA   NA   NA   NA   NA    0    0    0     0
## S   NA   NA   NA   NA   NA   NA    0    0    0     0
## T   NA   NA   NA   NA   NA   NA    0    0    0     0
## V   NA   NA   NA   NA   NA   NA    0    0    0     0
## W   NA   NA   NA   NA   NA   NA    0    0    0     0
## Y   NA   NA   NA   NA   NA   NA    0    0    0     0
```

Multiple alignments also inherit the `consensusString()` method from the `Biostrings` package. However, for more flexibility and consistency, we rather advise users to use the method `msaConsensusSequence()` method (see below).

6.2 Consensus Sequences and Conservation Scores

With version 1.7.1 of `msa`, new methods have been provided that allow for the computation of consensus sequences and conservation scores. By default, the `msaConsensusSequence()` method is a wrapper around the `consensusString()` method from the `Biostrings`:

```
printSplitString <- function(x, width=getOption("width") - 1)
{
    starts <- seq(from=1, to=nchar(x), by=width)

    for (i in 1:length(starts))
        cat(substr(x, starts[i], starts[i] + width - 1), "\n")
}

printSplitString(msaConsensusSequence(myFirstAlignment))

## ------------------------------------------------------------------
## -------------------------------------------------------------------?
## ?????????Y????D???????D??????R?Q???????????P?P???YTEEE??TW?TL??
## RQ??L????AC?E???G?----??LG???D?IPQLE?VSQ?LQ??TGWR???VPGL?P???FF?
## ?LA?R?FP?TQ?IR????LDY??EPDIFHELFGHVPLLSDP?FA?F?Q?YG?LA???A?????E
## ?LARLYW?TVEFGL????-???KAYGAGLLSSF?ELQYCLSD-?P???PF?LE??M?T?Y?ID?
## FQPLYFV??SF??L?E??R??D?T??????????P??????V?D?????????????????IL?
## ?A???---
```

However, there is also a second method for computing consensus sequence that has been implemented in line with a consensus sequence method implemented in TEXshade that allows for specify an upper and a lower conservation threshold (see example below). This method can be accessed via the argument `type="upperlower"`. Additional customizations are available, too:

```
printSplitString(msaConsensusSequence(myFirstAlignment, type="upperlower",
                                      thresh=c(40, 20)))

## ------------------------------------------------------------------
## -------------------------------------------------------------------.
## .........y....d.......d......r.q...........p.p...Yteee..tW.Tl..
## rq..l....Ac.e...g.----..lg...d.iPqle.vsq.Lq..TGwr...VpgL.p...Ff.
## .LA.r.Fp.tq.iR....ldY..EPDifHelfGHvPllsdp.FA.f.q.yG.la...a.....e
## .larlYW.TvEfGL....-...kayGaGllSSf.Elqyclsd-.p...pf.le..m.t.Y.id.
## fQplYfV..sf..l.e..r..d.t..........p......v.d................il.
## .a...---
```

Regardless of which method is used, masks are taken into account: masked rows/sequences are neglected and masked columns are shown as "#" in the consensus sequence:

```
printSplitString(msaConsensusSequence(myMaskedAlignment, type="upperlower",
                                      thresh=c(40, 20)))

## ################################################################
## ####################################--------------------------.
## .........y....d.......d......r.q...........p.p...Yteee..tW.Tl..
## rq..l....Ac.e...g.----..lg...d.iPqle.vsq.Lq..TGwr...VpgL.p...Ff.
## .LA.r.Fp.tq.iR....ldY..EPDifHelfGHvPllsdp.FA.f.q.yG.la...a.....e
## .larlYW.TvEfGL....-...kayGaGllSSf.Elqyclsd-.p...pf.le..m.t.Y.id.
## fQplYfV..sf..l.e..r..d.t.........p......v.d................il.
## .a...---
```

The main purpose of consensus sequences is to get an impression of conservation at individual positions/columns of a multiple alignment. The `msa` package also provides another means of analyzing conservation: the method `msaConservationScore()` computes sums of pairwise scores for a given substitution/scoring matrix. Thereby, conservation can also be analyzed in a more sensible way than by only taking relative frequencies of letters into account as `msaConsensusSequence()` does.

```
data(BLOSUM62)
msaConservationScore(myFirstAlignment, BLOSUM62)

##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -55  -95  -94 -109  -71  -71  -55  -71 -119 -121  -71  -95  -55
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -55  -71  -71  -39  -39  -39  -55  -55  -55  -71  -23  -71  -55
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -81  -39  -96  -69  -55  -39  -54  -71  -79  -71  -71  -71  -39
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -71  -71  -55  -55  -55  -71  -39  -71  -71  -71  -73  -71  -71
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -55  -71  -39  -55  -55  -39  -64  -77  -39  -71  -55   -7  -71
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -55  -71  -55  -23  -71  -55  -71  -97  -55  -39  -55  -23  -55
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -39  -39  -55  -91  -71  -39  -79  -55  -88 -121  -23 -103  -71
##    -    -    -    -    -    -    -    -    -    -    -    -    -
## -110  -87  -71  -71  -55 -119  -71  -55  -71  -39  -71  -39  -71
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -55  -71   -7  -55  -71  -71  -55  -39  -55  -87  -55  -79  -55
##    -    -    -    -    -    -    -    -    -    -    ?    ?    ?
##  -71  -23   41  -39  -23  -55  -55  -71  -55  -55  -59  -60 -113
##    ?    ?    ?    ?    ?    ?    ?    Y    ?    ?    ?    ?    D
##  -60  -75    9   44  -47  -59  -11   18  -45  -58  -71  -91   -1
##    ?    ?    ?    ?    ?    ?    ?    D    ?    ?    ?    ?    ?
```

```
##  -74  -66  -27  -42  -59  -27  -51  246   52   74   52   97   74
##    ?    R    ?    Q    ?    ?    ?    ?    ?    ?    ?    ?    ?
##   -5  100   77  125   60   51  -50  -99  -62  -36  -19  -32   49
##    ?    ?    ?    P    ?    P    ?    ?    ?    Y    T    E    E
##   96   49   -2  166   30  218    6   21   34  567  141  160  165
##    E    ?    ?    T    W    ?    T    L    ?    ?    R    Q    ?
##  325  179   71  216  891   38  405  204  208   79  165  109   42
##    ?    L    ?    ?    ?    ?    A    C    ?    E    ?    ?    ?
##   79  249  113  106   93  172  324  536  109  324  261   72  157
##    G    ?    -    -    -    -    ?    ?    L    G    ?    ?    ?
##   54  156  -23  -71  -71  -55  163   60  204  267  153  -19   60
##    D    ?    I    P    Q    L    E    ?    V    S    Q    ?    L
##  288  141  296  567  183  196  181  145  228  236  189   20  324
##    Q    ?    ?    T    G    W    R    ?    ?    ?    V    P    G
##  171   74  145  405  486  411  167  216   36  125  324  199  388
##    L    ?    P    ?    ?    ?    F    F    ?    ?    L    A    ?
##  324  216  199   86   86   42  486  214  125  108  324  324   92
##    R    ?    F    P    ?    T    Q    ?    I    R    ?    ?    ?
##  341   45  486  223  172  184  106  343  233  405  117  117  100
##    ?    L    D    Y    ?    ?    E    P    D    I    F    H    E
##  209   92  110  567  124  132  405  567  486  149  214  648  301
##    L    F    G    H    V    P    L    L    S    D    P    ?    F
##  186  214  486  648  249  567  249  196  108  239  175   27  486
##    A    ?    F    ?    Q    ?    Y    G    ?    L    A    ?    ?
##  324  213  387   90  286   70  199  486  -73    7  196    3   26
##    ?    A    ?    ?    ?    ?    ?    E    ?    L    A    R    L
##  124   83   76   29   49   40   71  108   71  292  261  165  244
##    Y    W    ?    T    V    E    F    G    L    ?    ?    ?    ?
##  567  891  301  405  244  405  439  486  324  173  157  129   97
##    -    ?    ?    ?    K    A    Y    G    A    G    L    L    S
##    6   92  143  140  285  124  502  486  262  486  244  217  324
##    S    F    ?    E    L    Q    Y    C    L    S    D    -    ?
##  324   52   93  405   77   81  451  261  276  171  183 -117  131
##    P    ?    ?    ?    P    F    ?    L    E    ?    ?    M    ?
##  436  104   45  137  163  214  254  175  131  175  120  149    8
##    T    ?    Y    ?    I    D    ?    F    Q    P    L    Y    F
##  165    0  567  102  288  190   73  388  405  169  108  567  382
##    V    ?    ?    S    F    ?    ?    L    ?    E    ?    ?    R
##  324  100  182  262  306  118   82  124   62  264   64   95  129
##    ?    ?    D    ?    T    ?    ?    ?    ?    ?    ?    ?    ?
##  -75  -10  134   92   87   44   68   51   56   18   41   44   61
##    ?    ?    P    ?    ?    ?    ?    ?    ?    V    ?    D    ?
##   79   83   91  161   56   69   51   12   42   66  -40   78  116
##    ?    ?    ?    ?    ?    ?    ?    ?    ?    ?    ?    ?    ?
##   43    1  -40  -71  -11  -86  -70  -67   37   -8  -41  -38  -11
##    ?    ?    ?    I    L    ?    ?    A    ?    ?    ?    -    -
```

```
##  -28  -87   -7   -5  -44    4 -110  -44  -84  -68  -74  -83  -55
##    -
##  -47
```

As the above example shows, a substitution matrix must be provided. The result is obviously a vector as long as the alignment has columns. The entries of the vector are labeled by the consensus sequence. The way the consensus sequence is computed can be customized:

```
msaConservationScore(myFirstAlignment, BLOSUM62, gapVsGap=0,
                     type="upperlower", thresh=c(40, 20))

##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -80 -120 -119 -134  -96  -96  -80  -96 -144 -146  -96 -120  -80
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -80  -96  -96  -64  -64  -64  -80  -80  -80  -96  -48  -96  -80
##    -    -    -    -    -    -    -    -    -    -    -    -    -
## -106  -64 -121  -94  -80  -64  -90  -96 -104  -96  -96  -96  -64
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -96  -96  -80  -80  -80  -96  -64  -96  -96  -96  -98  -96  -96
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -80  -96  -64  -80  -80  -64  -89 -102  -64  -96  -80  -32  -96
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -80  -96  -80  -48  -96  -80  -96 -122  -80  -64  -80  -48  -80
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -64  -64  -80 -116  -96  -64 -104  -80 -113 -146  -48 -128  -96
##    -    -    -    -    -    -    -    -    -    -    -    -    -
## -135 -112  -96  -96  -80 -144  -96  -80  -96  -64  -96  -64  -96
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -80  -96  -32  -80  -96  -96  -80  -64  -80 -112  -80 -104  -80
##    -    -    -    -    -    -    -    -    -    -    .    .    .
##  -96  -48   16  -64  -48  -80  -80  -96  -80  -80  -75  -76 -129
##    .    .    .    .    .    .    .    y    .    .    .    .    d
##  -76  -91    8   43  -48  -63  -15   14  -49  -62  -80 -100  -10
##    .    .    .    .    .    .    .    d    .    .    .    .    .
##  -90  -82  -43  -58  -75  -36  -60  246   52   74   52   97   74
##    .    r    .    q    .    .    .    .    .    .    .    .    .
##   -5  100   77  125   60   51  -66 -115  -71  -45  -28  -36   48
##    .    .    .    p    .    p    .    .    .    Y    t    e    e
##   95   48   -3  166   30  218    6   21   33  567  141  160  165
##    e    .    .    t    W    .    T    l    .    .    r    q    .
##  325  179   71  216  891   38  405  204  208   79  165  109   42
##    .    l    .    .    .    .    A    c    .    e    .    .    .
##   79  249  113  106   93  172  324  536  109  324  261   72  157
##    g    .    -    -    -    -    .    .    l    g    .    .    .
##   54  156  -48  -96  -96  -80  163   60  204  267  153  -20   60
##    d    .    i    P    q    l    e    .    v    s    q    .    L
```

```
## 288 141 296 567 183 196 181 145 228 236 189  20 324
##   q   .   .   T   G   w   r   .   .   .   V   p   g
## 171  74 145 405 486 411 167 216  36 125 324 199 388
##   L   .   p   .   .   .   F   f   .   .   L   A   .
## 324 216 199  86  86  42 486 214 125 108 324 324  92
##   r   .   F   p   .   t   q   .   i   R   .   .   .
## 341  45 486 223 172 184 106 343 233 405 117 117 100
##   .   l   d   Y   .   .   E   P   D   i   f   H   e
## 209  92 110 567 124 132 405 567 486 149 214 648 301
##   l   f   G   H   v   P   l   l   s   d   p   .   F
## 186 214 486 648 249 567 249 196 108 239 175  27 486
##   A   .   f   .   q   .   y   G   .   l   a   .   .
## 324 213 387  90 286  70 199 486 -89   7 196   3  26
##   .   a   .   .   .   .   .   e   .   l   a   r   l
## 124  83  76  29  48  40  71 108  71 292 261 165 244
##   Y   W   .   T   v   E   f   G   L   .   .   .   .
## 567 891 301 405 244 405 439 486 324 173 157 129  97
##   -   .   .   .   k   a   y   G   a   G   l   l   S
## -58  92 143 140 285 124 502 486 262 486 244 217 324
##   S   f   .   E   l   q   y   c   l   s   d   -   .
## 324  52  93 405  77  81 451 261 276 171 183 -142 131
##   p   .   .   .   p   f   .   l   e   .   .   m   .
## 436 104  45 137 163 214 254 175 131 175 120 149   8
##   t   .   Y   .   i   d   .   f   Q   p   l   Y   f
## 165   0 567 102 288 190  73 388 405 169 108 567 382
##   V   .   .   s   f   .   .   l   .   e   .   .   r
## 324 100 182 262 306 118  82 124  62 264  64  95 129
##   .   .   d   .   t   .   .   .   .   .   .   .   .
## -91 -10 134  92  87  44  68  51  56  18  41  44  61
##   .   .   p   .   .   .   .   .   .   v   .   d   .
##  79  83  90 161  56  69  51  12  42  65 -41  77 115
##   .   .   .   .   .   .   .   .   .   .   .   .   .
##  42   0 -44 -75 -15 -90 -74 -76  33 -12 -45 -42 -15
##   .   .   .   i   l   .   .   a   .   .   .   -   -
## -32 -91 -11  -9 -60 -12 -126 -60 -100 -84 -90 -108 -80
##   -
## -96
```

The additional argument `gapVsGap` allows for controlling how pairs of gap are taken into account when computing pairwise scores (see `?msaConservationScore` for more details).

Conservation scores can also be computed from masked alignments. For masked columns, `NA`'s are returned:

```
msaConservationScore(myMaskedAlignment, BLOSUM62, gapVsGap=0,
                     type="upperlower", thresh=c(40, 20))
```

```
##    #    #    #    #    #    #    #    #    #    #    #    #    #
##   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA
##    #    #    #    #    #    #    #    #    #    #    #    #    #
##   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA
##    #    #    #    #    #    #    #    #    #    #    #    #    #
##   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA
##    #    #    #    #    #    #    #    #    #    #    #    #    #
##   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA
##    #    #    #    #    #    #    #    #    #    #    #    #    #
##   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA
##    #    #    #    #    #    #    #    #    #    #    #    #    #
##   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA
##    #    #    #    #    #    #    #    #    #    #    #    #    #
##   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA   NA
##    #    #    #    #    #    #    #    #    #    -    -    -    -
##   NA   NA   NA   NA   NA   NA   NA   NA   NA  -64  -96  -64  -96
##    -    -    -    -    -    -    -    -    -    -    -    -    -
##  -80  -96  -32  -80  -96  -96  -80  -64  -80 -112  -80 -104  -80
##    -    -    -    -    -    -    -    -    -    -    .    .    .
##  -96  -48   16  -64  -48  -80  -80  -96  -80  -80  -75  -76 -129
##    .    .    .    .    .    .    .    y    .    .    .    .    d
##  -76  -91    8   43  -48  -63  -15   14  -49  -62  -80 -100  -10
##    .    .    .    .    .    .    .    d    .    .    .    .    .
##  -90  -82  -43  -58  -75  -36  -60  246   52   74   52   97   74
##    .    r    .    q    .    .    .    .    .    .    .    .    .
##   -5  100   77  125   60   51  -66 -115  -71  -45  -28  -36   48
##    .    .    .    p    .    p    .    .    .    Y    t    e    e
##   95   48   -3  166   30  218    6   21   33  567  141  160  165
##    e    .    .    t    W    .    T    l    .    .    r    q    .
##  325  179   71  216  891   38  405  204  208   79  165  109   42
##    .    l    .    .    .    .    A    c    .    e    .    .    .
##   79  249  113  106   93  172  324  536  109  324  261   72  157
##    g    .    -    -    -    -    .    .    l    g    .    .    .
##   54  156  -48  -96  -96  -80  163   60  204  267  153  -20   60
##    d    .    i    P    q    l    e    .    v    s    q    .    L
##  288  141  296  567  183  196  181  145  228  236  189   20  324
##    q    .    .    T    G    w    r    .    .    .    V    p    g
##  171   74  145  405  486  411  167  216   36  125  324  199  388
##    L    .    p    .    .    .    F    f    .    .    L    A    .
##  324  216  199   86   86   42  486  214  125  108  324  324   92
##    r    .    F    p    .    t    q    .    i    R    .    .    .
##  341   45  486  223  172  184  106  343  233  405  117  117  100
##    .    l    d    Y    .    .    E    P    D    i    f    H    e
##  209   92  110  567  124  132  405  567  486  149  214  648  301
##    l    f    G    H    v    P    l    l    s    d    p    .    F
##  186  214  486  648  249  567  249  196  108  239  175   27  486
```

```
##    A    .    f    .    q    .    y    G    .    l    a    .    .
##  324  213  387   90  286   70  199  486  -89    7  196    3   26
##    .    a    .    .    .    .    .    e    .    l    a    r    l
##  124   83   76   29   48   40   71  108   71  292  261  165  244
##    Y    W    .    T    v    E    f    G    L    .    .    .    .
##  567  891  301  405  244  405  439  486  324  173  157  129   97
##    -    .    .    .    k    a    y    G    a    G    l    l    S
##  -58   92  143  140  285  124  502  486  262  486  244  217  324
##    S    f    .    E    l    q    y    c    l    s    d    -    .
##  324   52   93  405   77   81  451  261  276  171  183 -142  131
##    p    .    .    .    p    f    .    l    e    .    .    m    .
##  436  104   45  137  163  214  254  175  131  175  120  149    8
##    t    .    Y    .    i    d    .    f    Q    p    l    Y    f
##  165    0  567  102  288  190   73  388  405  169  108  567  382
##    V    .    .    s    f    .    .    l    .    e    .    .    r
##  324  100  182  262  306  118   82  124   62  264   64   95  129
##    .    .    d    .    t    .    .    .    .    .    .    .    .
##  -91  -10  134   92   87   44   68   51   56   18   41   44   61
##    .    .    p    .    .    .    .    .    .    v    .    d    .
##   79   83   90  161   56   69   51   12   42   65  -41   77  115
##    .    .    .    .    .    .    .    .    .    .    .    .    .
##   42    0  -44  -75  -15  -90  -74  -76   33  -12  -45  -42  -15
##    .    .    .    i    l    .    .    a    .    .    .    -    -
##  -32  -91  -11   -9  -60  -12 -126  -60 -100  -84  -90 -108  -80
##    -
##  -96
```

6.3 Interfacing to Other Packages

There are also other sequence analysis packages that use or make use of multiple sequence alignments. The `msa` package does not directly interface to these packages in order to avoid dependencies and possible incompatibilities. However, `msa` provides a function `msaConvert()` that allows for converting multiple sequence alignment objects to other types/classes. Currently, five such conversions are available, namely to the classes `alignment` (`seqinr` package [2]), `align` (`bios2mds` package [14]), `AAbin/DNAbin` (`ape` package [10]), and `phyDat` (`phangorn` package [11]). Except for the conversion to the class `phyDat`, these conversion are performed without loading or depending on the respective packages.

In the following example, we perform a multiple alignment of Hemoglobin alpha example sequences and convert the result for later processing with the `seqinr` package:

```
hemoSeq <- readAAStringSet(system.file("examples/HemoglobinAA.fasta",
                                        package="msa"))
hemoAln <- msa(hemoSeq)

## use default substitution matrix
```

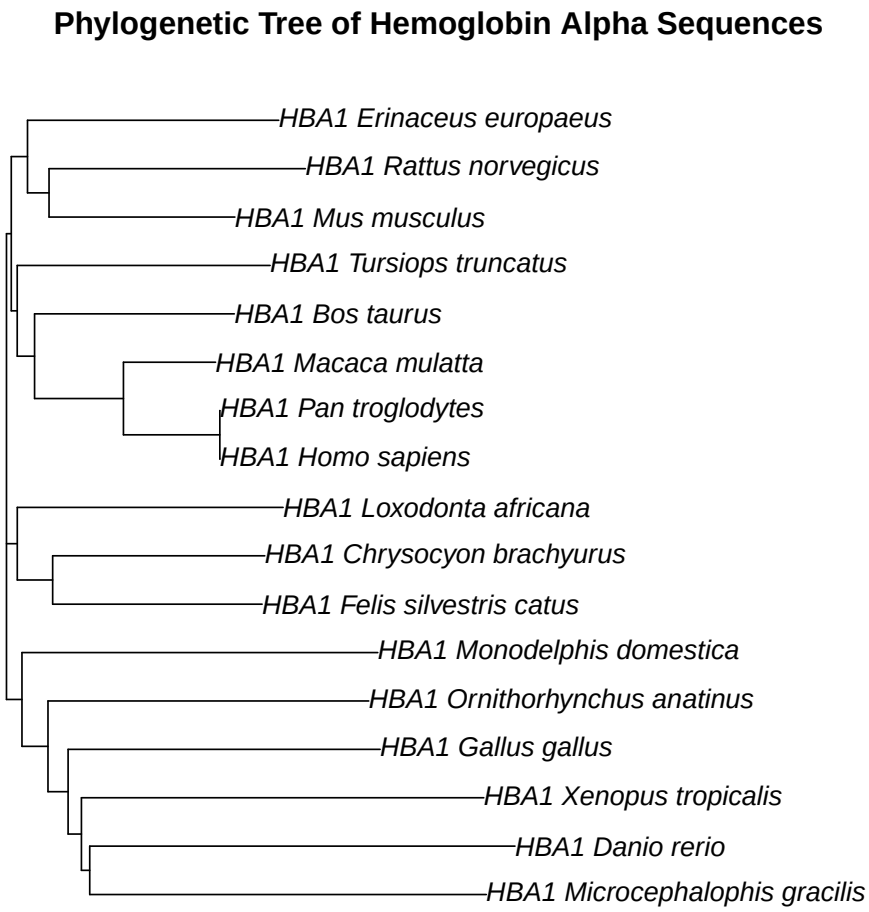
```
hemoAln

## CLUSTAL 2.1
##
## Call:
##    msa(hemoSeq)
##
## MsaAAMultipleAlignment with 17 rows and 143 columns
##      aln                                      names
##  [1] -VLSPADKTNVKAAWGKV...LDKFLASVSTVLTSKYR HBA1_Homo_sapiens
##  [2] MVLSPADKTNVKAAWGKV...LDKFLASVSTVLTSKYR HBA1_Pan_troglodytes
##  [3] -VLSPADKSNVKAAWGKV...LDKFLASVSTVLTSKYR HBA1_Macaca_mulatta
##  [4] -VLSAADKGNVKAAWGKV...LDKFLANVSTVLTSKYR HBA1_Bos_taurus
##  [5] -VLSPADKTNVKGTWSKI...LDKFLASVSTVLTSKYR HBA1_Tursiops_tru...
##  [6] -VLSGEDKSNIKAAWGKI...LDKFLASVSTVLTSKYR HBA1_Mus_musculus
##  [7] MVLSADDKTNIKNCWGKI...LDKFLASVSTVLTSKYR HBA1_Rattus_norve...
##  [8] -VLSATDKANVKTFWGKL...LDKFLATVATVLTSKYR HBA1_Erinaceus_eu...
##  [9] -VLSAADKSNVKACWGKI...LDKFFSAVSTVLTSKYR HBA1_Felis_silves...
## [10] -VLSPADKTNIKSTWDKI...LDKFFTAVSTVLTSKYR HBA1_Chrysocyon_b...
## [11] -VLSDNDKTNVKATWSKV...LDKFLSNVSTVLTSKYR HBA1_Loxodonta_af...
## [12] -VLSAADKTNVKAAWSKV...LDKFLALLSTVLTSKYR HBA1_Monodelphis_...
## [13] -MLTDAEKKEVTALWGKA...MDKFLSKVATVLTSKYR HBA1_Ornithorhync...
## [14] -VLSAADKNNVKGIFTKI...LDKFLCAVGTVLTAKYR HBA1_Gallus_gallus
## [15] -HLTADDKKHIKAIWPSV...LDKFLVSVSNVLTSKYR HBA1_Xenopus_trop...
## [16] -VLTEEDKARVRVAWVPV...VDKFLGQISKVLASRYR HBA1_Microcephalo...
## [17] -SLSDTDKAVVKAIWAKI...VDKFFNNLALALSEKYR HBA1_Danio_rerio
##  Con -VLS?ADK?NVKA?WGK?...LDKFLA?VSTVLTSKYR Consensus

hemoAln2 <- msaConvert(hemoAln, type="seqinr::alignment")
```

Now we compute a distance matrix using the `dist.alignment()` function from the `seqinr` package:

```
library(seqinr)

d <- dist.alignment(hemoAln2, "identity")
as.matrix(d)[2:5, "HBA1_Homo_sapiens", drop=FALSE]

##                          HBA1_Homo_sapiens
## HBA1_Pan_troglodytes            0.0000000
## HBA1_Macaca_mulatta             0.1684304
## HBA1_Bos_taurus                 0.3472281
## HBA1_Tursiops_truncatus         0.4038819
```

Now we can construct a phylogenetic tree with the neighbor joining algorithm using the `nj()` function from the `ape` package:

```
library(ape)

hemoTree <- nj(d)
plot(hemoTree, main="Phylogenetic Tree of Hemoglobin Alpha Sequences")
```



The following example shows how to convert a multiple alignment object in an object of class `align` as defined by the `bios2mds` package:

```
hemoAln3 <- msaConvert(hemoAln, type="bios2mds::align")
str(hemoAln3)

## List of 17
##  $ HBA1_Homo_sapiens            : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Pan_troglodytes         : chr [1:143] "M" "V" "L" "S" ...
```

```
##  $ HBA1_Macaca_mulatta           : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Bos_taurus               : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Tursiops_truncatus       : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Mus_musculus             : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Rattus_norvegicus        : chr [1:143] "M" "V" "L" "S" ...
##  $ HBA1_Erinaceus_europaeus      : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Felis_silvestris_catus   : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Chrysocyon_brachyurus    : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Loxodonta_africana       : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Monodelphis_domestica    : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Ornithorhynchus_anatinus : chr [1:143] "-" "M" "L" "T" ...
##  $ HBA1_Gallus_gallus            : chr [1:143] "-" "V" "L" "S" ...
##  $ HBA1_Xenopus_tropicalis       : chr [1:143] "-" "H" "L" "T" ...
##  $ HBA1_Microcephalophis_gracilis: chr [1:143] "-" "V" "L" "T" ...
##  $ HBA1_Danio_rerio              : chr [1:143] "-" "S" "L" "S" ...
##  - attr(*, "class")= chr "align"
```

The conversions to the standard `Biostrings` classes are straightforward using standard `as()` methods and not provided by the `msaConvert()` function. The following example converts a multiple alignment object to class `BStringSet` (e.g. the `msaplot()` function from the `ggtree` package [16] accepts `BStringSet` objects):

```
hemoAln4 <- as(hemoAln, "BStringSet")
hemoAln4
```

```
##   A BStringSet instance of length 17
##      width seq                                 names
##  [1]   143 -VLSPADKTNVKAAW...KFLASVSTVLTSKYR HBA1_Homo_sapiens
##  [2]   143 MVLSPADKTNVKAAW...KFLASVSTVLTSKYR HBA1_Pan_troglodytes
##  [3]   143 -VLSPADKSNVKAAW...KFLASVSTVLTSKYR HBA1_Macaca_mulatta
##  [4]   143 -VLSAADKGNVKAAW...KFLANVSTVLTSKYR HBA1_Bos_taurus
##  [5]   143 -VLSPADKTNVKGTW...KFLASVSTVLTSKYR HBA1_Tursiops_tru...
##  ...   ... ...
## [13]   143 -MLTDAEKKEVTALW...KFLSKVATVLTSKYR HBA1_Ornithorhync...
## [14]   143 -VLSAADKNNVKGIF...KFLCAVGTVLTAKYR HBA1_Gallus_gallus
## [15]   143 -HLTADDKKHIKAIW...KFLVSVSNVLTSKYR HBA1_Xenopus_trop...
## [16]   143 -VLTEEDKARVRVAW...KFLGQISKVLASRYR HBA1_Microcephalo...
## [17]   143 -SLSDTDKAVVKAIW...KFFNNLALALSEKYR HBA1_Danio_rerio
```

Note: The `msaConvert()` function has been introduced in version 1.3.3 of the `msa` package. So, to have this function available, at least Bioconductor 3.3 is required, which requires at least R 3.3.0.

7 Pretty-Printing Multiple Sequence Alignments

As already mentioned above, the `msa` package offers the function `msaPrettyPrint()` which allows for pretty-printing multiple sequence alignments using the LaTeX package TeXshade [1]. Which prerequisites are necessary to take full advantage of the `msaPrettyPrint()` function is described in Section 2.

The `msaPrettyPrint()` function writes a multiple sequence alignment to an alignment (`.aln`) file and then creates LaTeX code for pretty-printing the multiple sequence alignment on the basis of the LaTeX package TeXshade. Depending on the choice of the `output` argument, the function `msaPrettyPrint()` either prints a LaTeX fragment to the R session (choice `output="asis"`) or writes a LaTeX source file (choice `output="tex"`) that it processes to a DVI file (choice `output="dvi"`) or PDF file (choice `output="pdf"`). Note that no extra software is needed for choices `output="asis"` and `output="tex"`. For `output="dvi"` and `output="pdf"`, however, a TeX/LaTeX distribution must be installed in order to translate the LaTeX source file into the desired target format (DVI or PDF).

The function `msaPrettyPrint()` allows for making the most common settings directly and conveniently via an R interface without the need to know the details of LaTeX or TeXshade. In the following, we will describe some of these customizations. For all possibilities, the user is referred to the documentation of TeXshade.[5]

7.1 Consensus Sequence and Sequence Logo

The consensus sequence of the alignment is one of the most important results of a multiple sequence alignment. `msaPrettyPrint()` has a standard possibility to show this consensus sequence with the parameter `showConsensus`. The default value is `"bottom"`, which results in the following:

```
msaPrettyPrint(myFirstAlignment, output="asis", y=c(164, 213),
               subset=c(1:6), showNames="none", showLogo="none",
               consensusColor="ColdHot", showLegend=FALSE,
               askForOverwrite=FALSE)
```

```
IAYNYRHGQPIPRVEYTEEEKQTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYTEEERKTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYMEEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLL   213
IAYNYRHGQPIPRVEYTEEEKKTWGTVFRTLKSLYKTHACYEHNHIFPLL   212
.....DFTLPQPLDRYSAEDHATWATLYQRQCKLLPGRACDEFMEGL...    67
QTLRPDFTMEQPVHRYTAADHATWRTLYDRQEALLPGRACDEFLQGL...    83
**********!***!****  !!*!** *** !****!!*! *******
```

Consensus sequences can also be displayed on top of a multiple sequence alignment or omitted completely.

[5] https://www.ctan.org/pkg/texshade

In the above example, an exclamation mark '!' in the consensus sequence stands for a conserved letter, i.e. a sequence positions in which all sequences agree, whereas an asterisk '*' stands for positions in which there is a majority of sequences agreeing. Positions in which the sequences disagree are left blank in the consensus sequence. For a more advanced example how to customize the consensus sequence, see the example in Subsection 7.4 below.

The color scheme of the consensus sequence can be configured with the `consensusColors` parameter. Possible values are `"ColdHot"`, `"HotCold"`, `"BlueRed"`, `"RedBlue"`, `"GreenRed"`, `"RedGreen"`, or `"Gray"`. The above example uses the color scheme `"RedGreen"`.

Additionally, `msaPrettyPrint()` also offers a more sophisticated visual representation of the consensus sequence — sequence logos. Sequence logos can be displayed either on top of the multiple sequence alignment (`showLogo="top"`), below the multiple sequence alignment (`showLogo="bottom"`), or omitted at all (`showLogo="none"`):

```
msaPrettyPrint(myFirstAlignment, output="asis", y=c(164, 213),
               subset=c(1:6), showNames="none", showLogo="top",
               logoColors="rasmol", shadingMode="similar",
               showLegend=FALSE, askForOverwrite=FALSE)
```

```
IAYNYRHGQPIPRVEYTEEEKKQTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYTEEERKTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYMEEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLL   213
IAYNYRHGQPIPRVEYTEEEKKTWGTVFRTLKSLYKTHACYEHNHIFPLL   212
.....DFTLPQPLDRYSAEDHATWATLYQRQCKLLPGRACDEFMEGL...    67
QTLRPDFTMEQPVHRYTAADHATWRTLYDRQEALLPGRACDEFLQGL...    83
***********!***!***** !!*!****** !****!!*! *******
```

The color scheme of the sequence logo can be configured with the `logoColors` parameter. Possible values are `"chemical"`, `"rasmol"`, `"hydropathy"`, `"structure"`, `"standard area"`, and `"accessible area"`. The above example uses the color scheme `"rasmol"`.

Note that a consensus sequence and a sequence logo can be displayed together, but only on opposite sides.

Finally, a caveat: for computing consensus sequences, `msaPrettyPrint()` uses the functionality provided by TEXshade, therefore, the results need not match to the results of the methods described in Section 6 above.

7.2 Color Shading Modes

TEXshade offers different shading schemes for displaying the multiple sequence alignment itself. The following schemes are available: `"similar"`, `"identical"`, and `"functional"`. Moreover, there are five different color schemes available for shading: `"blues"`, `"reds"`, `"greens"`, `"grays"`, or `"black"`. The following example uses the shading mode `"similar"` along with the color scheme `"blues"`:

```
msaPrettyPrint(myFirstAlignment, output="asis", y=c(164, 213),
               showNames="none", shadingMode="similar",
               shadingColors="blues", showLogo="none",
               showLegend=FALSE, askForOverwrite=FALSE)
```

```
IAYNYRHGQPIPRVEYTEEEKQTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYTEEERKTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYMEEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLL   213
IAYNYRHGQPIPRVEYTEEEKKTWGTVFRTLKSLYKTHACYEHNHIFPLL   212
.....DFTLPQPLDRYSAEDHATWATLYQRQCKLLPGRACDEFMEGL...    67
QTLRPDFTMEQPVHRYTAADHATWRTLYDRQEALLPGRACDEFLQGL...    83
....PDWTIDQGWETYTQAEHDVWITLYERQTDMLHGRACDEFMRGL...    58
.........DNGFIHYPETEHQVWNTLITRQLKVIEGRACQEYLDGI...    50
.VCRADFTVAQDYD.YSDEEQAVWRTLCDRQTKLTRKLAHHSYLDGV...    65
          *** * !****  *!*!** **  * *  !* *** **
```

If the shading modes "similar" or "identical" are used, the shadingModeArg argument allows for setting a similarity threshold (a numerical value between 0 and 100). For shading mode "functional", the following settings of the shadingModeArg argument are possible: "charge", "hydropathy", "structure", "hemical", "rasmol", "standard area", and "accessible area". The following example uses shading mode "functional" along with shadingModeArg set to "structure":

```
msaPrettyPrint(myFirstAlignment, output="asis", y=c(164, 213),
               showNames="none", shadingMode="functional",
               shadingModeArg="structure",
               askForOverwrite=FALSE)
```

```
IAYNYRHGQPIPRVEYTEEEKQTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYTEEERKTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYMEEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLL   213
IAYNYRHGQPIPRVEYTEEEKKTWGTVFRTLKSLYKTHACYEHNHIFPLL   212
.....DFTLPQPLDRYSAEDHATWATLYQRQCKLLPGRACDEFMEGL...    67
QTLRPDFTMEQPVHRYTAADHATWRTLYDRQEALLPGRACDEFLQGL...    83
....PDWTIDQGWETYTQAEHDVWITLYERQTDMLHGRACDEFMRGL...    58
.........DNGFIHYPETEHQVWNTLITRQLKVIEGRACQEYLDGI...    50
.VCRADFTVAQDYD.YSDEEQAVWRTLCDRQTKLTRKLAHHSYLDGV...    65
```

```
X  external
X  ambivalent
X  internal
```

In the above example, a legend is shown that specifies the meaning of the color codes with which the letters are shaded. In some of the other examples above, we have suppressed this legend with the option showLegend=FALSE. The default, however, is that a legend is printed underneath the multiple sequence alignment like in the previous example.

7.3 Subsetting

In case that not the complete multiple sequence alignment should be printed, `msaPrettyPrint()` offers two ways of sub-setting. On the one hand, the `subset` argument allows for selecting only a subset of sequences. Not surprisingly, `subset` must be a numeric vector with indices of sequences to be selected. On the other hand, it is also possible to slice out certain positions of the multiple sequence alignment using the `y` argument. In the simplest case, `y` can be a numeric vector with two elements in ascending order which correspond to the left and right bounds between which the multiple sequence alignment should be displayed. However, it is also possible to slice out multiple windows. For this purpose, the argument `y` must be an `IRanges` object containing the starts and ends of the windows to be selected.

7.4 Additional Customizations

The `msaPrettyPrint()` function provides an interface to the most common functionality of TEXshade in a way that the user does not need to know the specific commands of TEXshade. TEXshade, however, provides a host of additional customizations many of which are not covered by the interface of the `msaPrettyPrint()` function. In order to allow users to make use of all functionality of TEXshade, `msaPrettyPrint()` offers the `furtherCode` argument through which users can add LATEX code to the `texshade` environment that is created by `msaPrettyPrint()`. Moreover, the `code` argument can be used to bypass all of `msaPrettyPrint()`'s generation of TEXshade code.

Here is an example how to use the `furtherCode` argument in order to customize the consensus sequence and to show a ruler on top:

```
msaPrettyPrint(myFirstAlignment, output="asis", y=c(164, 213),
               subset=c(1:6), showNames="none", showLogo="none",
               consensusColor="ColdHot", showLegend=FALSE,
               shadingMode="similar", askForOverwrite=FALSE,
               furtherCode=c("\\defconsensus{.}{lower}{upper}",
                             "\\showruler{1}{top}"))
```

```
        170       180       190       200       210
IAYNYRHGQPIPRVEYTEEEKQTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYTEEERKTWGTVFRTLKALYKTHACYEHNHIFPLL   213
IAYNYRHGQPIPRVEYMEEEKKTWGTVFKTLKSLYKTHACYEYNHIFPLL   213
IAYNYRHGQPIPRVEYTEEEKKTWGTVFRTLKSLYKTHACYEHNHIFPLL   212
.....DFTLPQPLDRYSAEDHATWATLYQRQCKLLPGRACDEFMEGL...    67
QTLRPDFTMEQPVHRYTAADHATWRTLYDRQEALLPGRACDEFLQGL...    83
iaynyrhgqpiPrveYteeek.TWgTvfrtlk.LykthACyE.nhifpll
```

7.5 Sweave or knitr Integration

The function `msaPrettyPrint()` is particularly well-suited for pretty-printing multiple alignments in Sweave [6] or knitr [15] documents. The key is to set `output` to `"asis"` when calling

msaPrettyPrint() and, at the same time, to let the R code chunk produce output that is directly included in the resulting LaTeX document as it is. This can be accomplished with the code chunk option results="tex" in Sweave and with the code chunk option results="asis" in knitr. Here is an example of a Sweave code chunk that displays a pretty-printed multiple sequence alignment inline:

```
<<AnyChunkName,results="tex">>=
msaPrettyPrint(myFirstAlignment, output="asis")
@
```

The same example in knitr:

```
<<AnyChunkName,results="asis">>=
msaPrettyPrint(myFirstAlignment, output="asis")
@
```

Note that, for processing the resulting LaTeX source document, the TeXshade package must be installed (see Section 2) and the TeXshade package must be loaded in the preamble:

```
\usepackage{texshade}
```

7.6 Sequence Names

The Biostrings package does not impose any restrictions on the names of sequences. Consequently, msa also allows all possible ASCII strings as sequence (row) names in multiple alignments. As soon as msaPrettyPrint() is used for pretty-printing multiple sequence alignments, however, the sequence names are interpreted as plain LaTeX source code. Consequently, LaTeX errors may arise because of characters or words in the sequence names that LaTeX does not or cannot interpret as plain text correctly. This particularly includes appearances of special characters and backslash characters in the sequence names.

The msa package offers a function msaCheckNames() which allows for finding and replacing potentially problematic characters in the sequence names of multiple alignment objects (see ?msaCheckNames). However, the best solution is to check sequence names carefully and to avoid problematic sequence names from the beginning. Note, moreover, that too long sequence names will lead to less appealing outputs, so users are generally advised to consider sequence names carefully.

7.7 Pretty-Printing Wide Alignments

If the alignment to be printed with msaPrettyPrint() is wide (thousands of columns or wider), LaTeX may terminate prematurely because of exceeded TeX capacity. Unfortunately, this problem remains opaque to the user, since texi2dvi() and texi2pdf() do not convey much details about LaTeX problems when typesetting a document. We recommend the following if a user encounters problems with running msaPrettyPrint()'s output with texi2dvi() and texi2pdf():

1. Run `pdflatex` on the generated `.tex` file to see whether it is actually a problem with TEX capacity.
2. If so, split the alignment into multiple chunks and run `msaPrettyPrint()` on each chunk separately.

The following example demonstrates this approach for a multiple aligment object '`aln`':

```
chunkSize <- 300 ## how much fits on one page depends on the length of
                 ## names and the number of sequences;
                 ## change to what suits your needs

for (start in seq(1, ncol(aln), by=chunkSize))
{
    end <- min(start + chunkSize - 1, ncol(aln))
    alnPart <- DNAMultipleAlignment(subseq(unmasked(aln), start, end))

    msaPrettyPrint(x=alnPart, output="pdf", subset=NULL,
                   file=paste0("aln_", start, "-", end, ".pdf"))
}
```

This creates multiple PDF files all of which show one part of the alignment. Please note, however, that the numbering of columns is restarted for each chunk.

7.8 Further Caveats

- Note that `texi2dvi()` and `texi2pdf()` always save the resulting DVI/PDF files to the current working directory, even if the LATEX source file is in a different directory. That is also the reason why the temporary file is created in the current working directory in the example below.
- TEXshade has a wide array of functionalities. Only the most common ones have been tested for interoperability with R. So the use of the arguments `furtherCode` and `code` is the user's own risk!

8 Known Issues

Memory Leaks

The original implementations of ClustalW, ClustalOmega, and MUSCLE are stand-alone command line programs which are only run once each time a multiple sequence alignment is performed. During the development of the `msa` package, we performed memory management checks using Valgrind [8] and discovered multiple memory leaks in ClustalW and MUSCLE. These memory leaks have no effect for the command line tools, since the program is closed each time the alignment is finished. In the implementation of the `msa` package, however, these memory leaks may have an effect if the same algorithm is run multiple times.

For MUSCLE, we managed to eliminate all memory leaks by deactivating the two parameters `weight1` and `weight2`. ClustalOmega did not show any memory leaks. ClustalW indeed has several memory leaks which are benign if the algorithm is run only a few times, but which may have more severe effects if the algorithm is run many times. ClustalOmega also has a minor memory leak, but the loss of data is so small that no major problems are to be expected except for thousands of executions of ClustalOmega.

ClustalOmega vs. Older GCC Versions on Linux/Unix

We have encountered peculiar behavior of ClustalOmega if the package was built using an older GCC version: if we built the package on an `x86_64` Linux system with GCC 4.4.7, ClustalOmega built smoothly and could be executed without any errors. However, the resulting multiple sequence alignment was more than sub-optimal. We could neither determine the source of this problem nor which GCC versions show this behavior. We therefore recommend Linux/Unix users to use an up-to-date GCC version (we used 4.8.2 during package development, which worked nicely) or, in case they encounter dubious results, to update to a newer GCC version and re-install the package.

ClustalOmega: OpenMP Support on Mac OS

ClustalOmega is implemented to make use of OpenMP (if available on the target platform). Due to issues on one of the Bioconductor build servers running Mac OS, we had to deactivate OpenMP generally for Mac OS platforms. If a Mac OS user wants to re-activate OpenMP, he/she should download the source package tarball, untar it, comment/uncomment the corresponding line in `msa/src/ClustalOmega/msaMakefile` (see first six lines), and build/install the package from source.

Build/installation issues

Some users have reported compiler and linker errors when building `msa` from source on Linux systems. In almost all cases, these could have been tracked down to issues with the `R` setup on those systems (e.g. a `Rprofile.site` file that makes changes to the `R` environment that are not compatible with `msa`'s Makefiles).[6] In most cases, these issues can be avoided by installing `msa` in a "vanilla `R` session", i.e. starting `R` with option `--vanilla` when installing `msa`.

9 Future Extensions

We envision the following changes/extensions in future versions of the package:

- Integration of more multiple sequence alignment algorithms, such as, T-Coffee [9] or DIALIGN [7]
- Support for retrieving guide trees from the multiple sequence alignment algorithms

[6] See, e.g., `https://support.bioconductor.org/p/90735/`

- Interface to methods computing phylogenetic trees (e.g. as contained in the original implementation of ClustalW)
- Elimination of memory leaks described in Section 8 and re-activation of parameters that have been deactivated in order to avoid memory leaks
- More tolerant handling of custom substitution matrices (MUSCLE interface)

10 How to Cite This Package

If you use this package for research that is published later, you are kindly asked to cite it as follows:

> U. Bodenhofer, E. Bonatesta, C. Horejš-Kainrath, and S. Hochreiter (2015). msa: an R package for multiple sequence alignment. *Bioinformatics* **31**(24):3997–3999. DOI: bioinformatics/btv494.

To obtain a BibTEX entries of the reference, enter the following into your R session:

```
toBibtex(citation("msa"))
```

Moreover, we insist that, any time you cite the package, you also cite the original paper in which the original algorithm has been introduced (see bibliography below).

11 Change Log

Version 1.7.2:

- fix for new `clang` 4 compiler on Mac OS

Version 1.7.1:

- additional conversions implemented for `msaConvert()` function
- added a new method `msaConsensusSequence()` that extends the functionality provided by `Biostring`'s `consensusString()` method
- added a new method `msaConservationScore()`
- `print()` method extended such that it now also allows for customization of the consensus sequence (via the new `msaConsensusSequence()` method)
- package now depends on `Biostrings` version $\geq$2.40.0 in order to make sure that `consensusMatrix()` also works correctly for masked alignments
- corresponding changes in documentation and vignette

Version 1.7.0: new branch for Bioconductor 3.5 devel

Version 1.6.0: release as part of Bioconductor 3.4

Version 1.5.5:

- fixes in ClustalOmega source code to ensure Windows compatibility of GCC6 compatibility fix

Version 1.5.4:

- bug fix in `msaClustalW()`: unsupported parameter '`tree`' deactivated
- fixes in ClustalOmega source code to ensure GCC 6 compatibility
- fix in `msaConvert()` function to improve safety of call to suggested package `phangorn`

Version 1.5.3:

- additional conversions implemented for `msaConvert()` function
- corresponding changes in documentation

Versions 1.5.1 and 1.5.2: version number bumps for technical reasons related to Bioconductor build servers

Version 1.5.0: new branch for Bioconductor 3.4 devel

Version 1.4.0: release as part of Bioconductor 3.3

Version 1.3.7:

- fixes in `msaPrettyPrint()` function

Version 1.3.6:)

- `msaPrettyPrint()` now also accepts dashes in file names
- added section about pretty-printing wide alignments to package vignette

Version 1.3.5:

- adaptation of displaying help text by `msa()` function

Version 1.3.4:

- added function for checking and fixing sequence names for possibly problematic characters that could lead to LaTeX errors when using `msaPrettyPrint()`
- corresponding changes in documentation
- minor namespace fix

Version 1.3.3:

- added function for converting multiple sequence alignments for use with other sequence alignment packages
- corresponding changes in documentation

Version 1.3.2:

- further fixes in Makefiles and Makevars files to account for changes in build system

- update of citation information

Version 1.3.1:

- fixes in Makefiles and Makevars files to account for changes in build system

Version 1.3.0: new branch for Bioconductor 3.3 devel

Version 1.2.0: release as part of Bioconductor 3.2

Version 1.1.3:

- bug fix related to custom substitution matrices in the MUSCLE interface
- corrections and updates of documentation

Version 1.1.2:

- new `print()` function for multiple alignments that also allows for displaying alignments in their entirety (plus additional customizations)
- strongly improved handling of custom substitution matrices by `msaClustalW()`: now custom matrices can also be supplied for nucleotide sequences which can also be passed via the `substitutionMatrix` argument. The `dnamatrix` argument is still available for the sake of backwards compatibility.
- strongly improved handling of custom substitution matrices by `msaMuscle()`
- fix of improperly aligned sequence logos produced by `msaPrettyPrint()`
- updated citation information

Version 1.1.1:

- fix of `msa()` function

Version 1.1.0: new branch for Bioconductor 3.2 devel

Version 1.0.0: first official release as part of Bioconductor 3.1

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