SeqinR 1.0−6

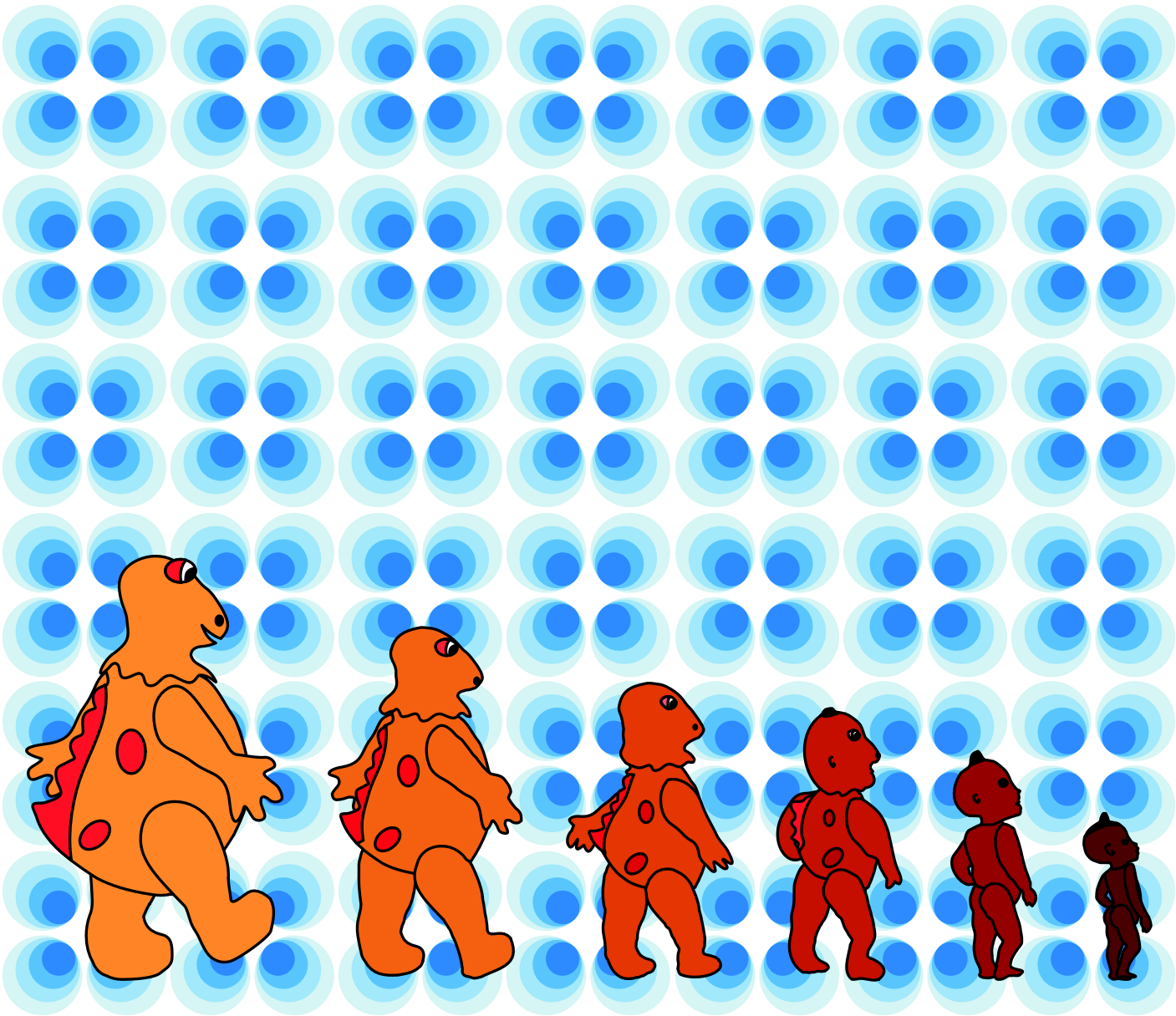


Figure 1: The march of progress icon is very common in popular press. This example is from page 46 of a 1984 summer issue of the tchek edition of *Playboy*.

The march of progress icon

The cover, an artwork created[1] by Lionel Humblot, is an allusion to what Stephen J. Gould considered as a caonical icon of "[t]he most serious and pervasive of all misconceptions about evolution equates the concept with some notion of progress, usually inherent and predictable, and leading to a human pinnacle" [14]. Some examples of the so-called "march of progress icon" out of hundreds in S.J. Gould's collection from popular press are given in the begining of his famous book *Wonderful life* [13].

Note that the underlying conception predates Darwin [37]. We know now that evolution doesn not equal progress, and this is illutrated here in the cover by the unusual **decreasing** size from the initial character (on the left) to the last one (on the right).

L'île aux enfants.

The character on the left

The character on the left is called Casimir, the cult character of the french TV show *l'île aux enfants* (literally Kid's island, a french adaptation of *Sesame Street* from 1974 to 1975 and then an autonomous production until 1982 when it eventually ended). Casimir was a muppet, human-sized, with an actor playing inside, representing an orange dinosaur (the exact taxonomy has never been published) with yellow and red spots. Casimir was symbolically chosen here for two reasons. Fisrt, it's birth correspond to one of the earliest paper from our

[1] with Canvas from ACD Systems.

lab about molecular evolution [16]. If you dig into **seqinR** you will find that the data from this more than 30 years old paper are still available[2]:

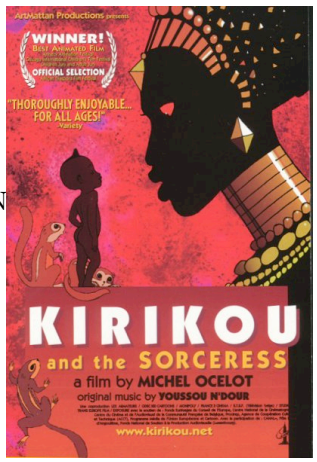
```
data(aaindex)
grth <- which(sapply(aaindex, function(x) length(grep("Grantham",
    x$A)) != 0))
lapply(aaindex[grth], "[[", "D")
```

```
$GRAR740101
[1] "Composition (Grantham, 1974)"

$GRAR740102
[1] "Polarity (Grantham, 1974)"

$GRAR740103
[1] "Volume (Grantham, 1974)"
```

Second, Casimir's life span correspond more or less to the time during which the sequence analysis software called ANALSEQ[3] [22] was under development in our lab. ANALSEQ has never been published as a regular paper (although it is mentioned in one of the ACNUC paper [15]), there is only a reference manual in french [22] also available on-line at http://biomserv.univ-lyon1.fr/doclogi/docanals/manuel.html. ANALSEQ was entirely written in FORTRAN 77, and although you won't find any fossil code from it within **seqinR**, we wanted to credit symbolically ANALSEQ as a kind of spiritual ancestor of **seqinR** with the cover.



Kirikou and the sorceress, a film by Michel Ocelot with original music by Youssou N'Dour.

The character on the right

The character on the right is called Kirikou. He is the main character of the animated film *Kirikou et la sorcière* (Kirikou and the sorceress, 1998) and *Kirikou et les bêtes sauvages* (Kirikou and the Wild Beasts, 2005). Kirikou was chosen as a symbol of **seqinR** development time. **SeqinR** started in september 2002 as part of the work of Delphine Charif's master of sciences. The first public presentation of **seqinR** was a seminar (2-JUL-2003, Lausanne University, Swiss) and the first public release on the CRAN[4] was in october 2004.

Technical details

The cover was saved from Canvas into an EPS[5] file. This file was then manually edited to remove non-ASCII characters. It was then converted into RGML[6] format with the following code based on `grid` [46], `XML` [5] and `grImport` [39]:

```
library(grid)
library(XML)
library(grImport)
PostScriptTrace("../figs/couverture.eps", "../figs/couverture.rgml")
```

[2] thanks to `aaindex` database [26, 56, 40].
[3] not to be confused with the ANALYSEQ program by Rodger Staden [53].
[4] Comprehensive R Archive Network.
[5] Encapsulated Postscrit.
[6] RDF (Resource Description Framework) Graph Modeling Language (http://www.cs.rpi.edu/~puninj/RGML/).

The picture was then edited to add automatically the current **seqinR** release number:

```
cover <- readPicture("../figs/couverture.rgml")
pdf(file = "../figs/cover.pdf", width = 21/2.54, height = 29.7/2.54)
pushViewport(plotViewport(margins = c(0, 0, 0, 0)))
grid.rect()
grid.picture(cover)
grid.text(paste("SeqinR", packageDescription("seqinr")$Version),
    gp = gpar(cex = 5), y = unit(0.72, "npc"))
popViewport()
dev.off()
```

And finally inserted at the begining of the LaTeX file with:

```
\atxy(0cm,0cm){
  \includegraphics[width=\paperwidth,height=\paperheight]{../figs/cover}
}
```

Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, grid, methods, stats, utils
- Other packages: MASS 7.2-27.1, XML 0.99-8, ade4 1.4-1, ape 1.8-2, gee 4.13-10, grImport 0.2, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

SeqinR 1.0-6: a contributed package to the R project for statistical computing devoted to biological sequences retrieval and analysis

Charif, D. Humblot, L. Lobry, J.R. Palmeira, L.

September 1, 2006

CONTENTS

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Licence

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Using and contributing

If you want to re-use or contribute to this document, some indications are given in the appendix (*under construction*)

CHAPTER 1

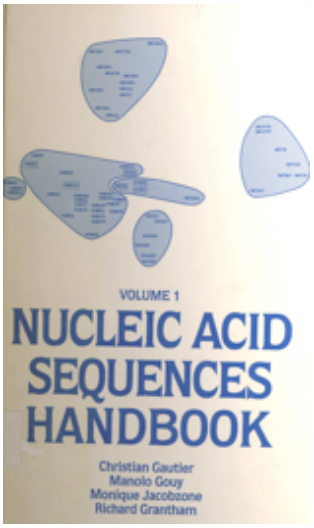
Introduction

Lobry, J.R.

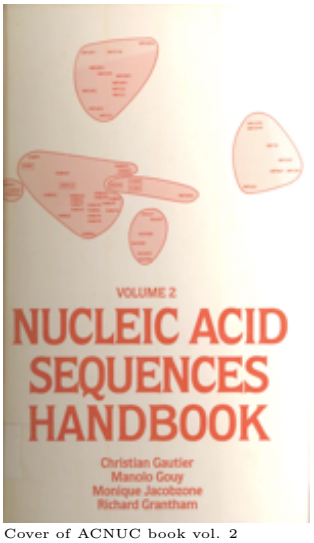
Contents

1.1 About ACNUC

ACNUC[1] was first a database of nucleic acids developed in the early 80's in the same lab (Lyon, France) that issued **seqinR**. ACNUC was first published as a printed book in two volumes [11, 12] whose covers are reproduced in margin there. At about the same time, two other databases were created, one in the USA (GenBank, at Los Alamos and now managed by the NCBI[2]), and another one in Germany (created in Köln by K. Stüber). To avoid duplication of efforts at the european level, a single repository database was initiated in Germany yielding the EMBL[3] database that moved from Köln to Heidelberg, and then to its current location at the EBI[4] near Cambridge. The DDBJ[5] started in 1986



Cover of ACNUC book vol. 1



Cover of ACNUC book vol. 2

[1] A contraction of ACides NUCléiques, that is *NUCleic ACids* in french (http://pbil.univ-lyon1.fr/databases/acnuc/acnuc.html)
[2] National Center for Biotechnology Information
[3] European Molecular Biology Laboratory
[4] European Bioinformatic Institute
[5] DNA Data Bank of Japan

at the NIG[6] in Mishima. These three main repository DNA databases are now collaborating to maintain the INSD[7] and are sharing data on a daily basis.

The sequences present in the ACNUC books [11, 12] were all the published nucleic acid sequences of about 150 or more continuous unambiguous nucleotides up to May or June 1981 from the journal given in table 1.1.

Journal name
Biochimie
Biochemistry (ACS)
Cell
Comptes Rendus de l'Académie des Sciences, Paris
European Journal of Biochemistry
FEBS Letters
Gene
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Molecular Biology
Molecular and General Genetics
Nature
Nucleic Acids Research
Proceedings of the National Academy of Sciences of the United States of America
Science

Table 1.1: The list of journals that were manually scanned for nucleic sequences that were included in the ACNUC books [11, 12]

ACNUC books are about 4.5 cm width

The total number of base pair was 474,439 in the two books. They were about 4.5 cm width. We can then compute of much place would it take to print the last GenBank release with the same format as the ACNUC book:

```
acnucbooksize <- 4.5
acnucbp <- 474439
mybank <- choosebank("genbank")
mybank$details
```

```
[1] "GenBank Rel. 155 (15 August 2006) Last Updated: Sep  1, 2006"
[2] "66,009,892,298 bases; 61,704,148 sequences; 3,242,595 subseqs; 435,242 refers."
[3] "Software by M. Gouy, Lab. Biometrie et Biologie Evolutive, Universite Lyon I"
```

```
bpbk <- unlist(strsplit(mybank$details[2], split = " "))[1]
bpbk
```

```
[1] "66,009,892,298"
```

```
bpbk <- as.numeric(paste(unlist(strsplit(bpbk, split = ",")),
    collapse = ""))
widthcm <- acnucbooksize * bpbk/acnucbp
(widthkm <- widthcm/10^5)
```

```
[1] 6.260963
```

It would be about 6.3 kilometer long in ACNUC book format to print to print GenBank today (September 1, 2006).

[6] National Institute of Genetics

[7] International Nucleotide Sequence Database (http://www.insdc.org/)

1.2 About R and CRAN

R [21, 47] is a *libre* language and environment for statistical computing and graphics which provides a wide variety of statistical and graphical techniques: linear and nonlinear modelling, statistical tests, time series analysis, classification, clustering, etc. Please consult the R project homepage at `http://www.R-project.org/` for further information.

The Comprehensive R Archive Network, CRAN, is a network of servers around the world that store identical, up-to-date, versions of code and documentation for R. At compilation time of this document, there were 63 mirrors available from 26 countries. Please use the CRAN mirror nearest to you to minimize network load, they are listed at `http://cran.r-project.org/mirrors.html`, and can be directly selected with the function `chooseCRANmirror()`.

1.3 About this document

In the terminology of the R project [21, 47], this document is a package *vignette*. The examples given thereafter were run under `Version 2.3.1 (2006-06-01)` on Fri Sep 1 23:35:01 2006 with Sweave [31]. The last compiled version of this document is distributed along with the **seqinR** package in the `/doc` folder. Once **seqinR** has been installed, the full path to the package is given by the following R code :

```
.find.package("seqinr")
```

```
[1] "/Users/lobry/Library/R/library/seqinr"
```

1.4 About sequin and seqinR

Sequin is the well known sofware used to submit sequences to GenBank, **seqinR** has definitively no connection with sequin. **seqinR** is just a shortcut, with no google hit, for "Sequences in R".

However, as a mnemotechnic tip, you may think about the **seqinR** package as the **R**eciprocal function of sequin: with sequin you can submit sequences to Genbank, with **seqinR** you can **R**etrieve sequences from Genbank. This is a very good summary of a major functionality of the **seqinR** package: to provide an efficient access to sequence databases under R.

1.5 About getting started

You need a computer connected to the Internet. First, install R on your computer. There are distributions for Linux, Mac and Windows users on the CRAN (`http://cran.r-project.org`). Then, install the `ape`, `ade4` and `seqinr` packages. This can be done directly in an R console with for instance the command `install.packages("seqinr")`. Last, load the **seqinR** package with:

```
library(seqinr)
```

The command `lseqinr()` lists all what is defined in the package **seqinR**:

```
lseqinr()[1:9]
```

```
[1] "AAstat"      "EXP"         "GC"          "GC1"         "GC2"
[6] "GC3"         "SEQINR.UTIL" "a"           "aaa"
```

We have printed here only the first 9 entries because they are too numerous. To get help on a specific function, say `aaa()`, just prefix its name with a question mark, as in `?aaa` and press enter.

1.6 About running R in batch mode

Although R is usually run in an interactive mode, some data pre-processing and analyses could be too long. You can run your R code in batch mode in a shell with a command that typically looks like :

```
unix$ R CMD BATCH input.R results.out &
```

where `input.R` is a text file with the R code you want to run and `results.out` a text file to store the outputs. Note that in batch mode, the graphical user interface is not active so that some graphical devices (*e.g.* `x11`, `jpeg`, `png`) are not available (see the R FAQ [19] for further details).

It's worth noting that R uses the XDR representation of binary objects in binary saved files, and these are portable across all R platforms. The `save()` and `load()` functions are very efficient (because of their binary nature) for saving and restoring any kind of R objects, in a platform independent way. To give a striking real example, at a given time on a given platform, it was about 4 minutes long to import a numeric table with 70000 lines and 64 columns with the defaults settings of the `read.table()` function. Turning it into binary format, it was then about 8 *seconds* to restore it with the `load()` function. It is therefore advisable in the `input.R` batch file to save important data or results (with something like `save(mybigdata, file = "mybigdata.RData")`) so as to be able to restore them later efficiently in the interactive mode (with something like `load("mybigdata.RData")`).

1.7 About the learning curve

Introduction

If you are used to work with a purely graphical user interface, you may feel frustrated in the beginning of the learning process because apparently simple things are not so easily obtained (*ce n'est que le premier pas qui coûte !*). In the long term, however, you are a winner for the following reasons.

1.7.1 Wheel (the)

Do not re-invent (there's a patent [27] on it anyway). At the compilation time of this document there were 798 contributed packages available. Even if you don't want to be spoon-feed *à bouche ouverte*, it's not a bad idea to look around there just to check what's going on in your own application field. Specialists all around the world are there.

1.7.2 Hotline

There is a very reactive discussion list to help you, just make sure to read the posting guide there: http://www.R-project.org/posting-guide.html before posting. Because of the high traffic on this list, we strongly suggest to answer *yes* at the question *Would you like to receive list mail batched in a daily digest?* when subscribing at https://stat.ethz.ch/mailman/listinfo/r-help. Some *bons mots* from the list are archived in the fortunes package.

1.7.3 Automation

Consider the 178 pages of figures in the additional data file 1 (http://genomebiology.com/2002/3/10/research/0058/suppl/S1) from [36]. They were produced in part automatically (with a proprietary software that is no more maintained) and manually, involving a lot of tedious and repetitive manipulations (such as italicising species names by hand in subtitles). In few words, a waste of time. The advantage of the environment is that once you are happy with the outputs (including graphical outputs) of an analysis for species x, it's very easy to run the same analysis on n species.

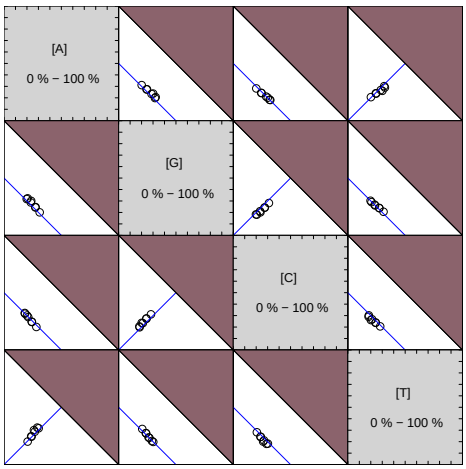
1.7.4 Reproducibility

If you do not consider the reproducibility of scientific results to be a serious problem in practice, then the paper by Jonathan Buckheit and David Donoho [2] is a must read. Molecular data are available in public databases, this is a necessary but not sufficient condition to allow for the reproducibility of results. Publishing the R source code that was used in your analyses is a simple way to greatly facilitate the reproduction of your results at the expense of no extra cost. At the expense of a little extra cost, you may consider to set up a RWeb server so that even the laziest reviewer may reproduce your results just by clicking on the "do it again" button in his web browser (*i.e.* without installing any software on his computer). For an example involving the **seqinR** pacakage, follow this link http://pbil.univ-lyon1.fr/members/lobry/repro/bioinfo04/ to reproduce on-line the results from [3].

1.7.5 Fine tuning

You have full control on everything, even the source code for all functions is available. The following graph was specifically designed to illustrate the first experimental evidence [48] that, on average, we have also [A]=[T] and [C]=[G] in single-stranded DNA. These data from Chargaff's lab give the base composition of the L (Ligth) strand for 7 bacterial chromosomes.

```
example(chargaff)
```



This is a very specialised graph. The filled areas correspond to non-allowed values beause the sum of the four bases frequencies cannot exceed 100 %. The white areas correspond to possible values (more exactly to the projection from $\mathbb{R}^4$ to the corresponding $\mathbb{R}^2$ planes of the region of allowed values). The lines correspond to the very small subset of allowed values for which we have in addition [A]=[T] and [C]=[G]. Points represent observed values in the 7 bacterial chromosomes. The whole graph is entirely defined by the code given in the example of the `chargaff` dataset (`?chargaff` to see it).

Another example of highly specialised graph is given by the function `tablecode()` to display a genetic code as in textbooks :

```
tablecode()
```

Genetic code 1 : standard

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

It's very convenient in practice to have a genetic code at hand, and moreover here, all genetic code variants are available :

```
tablecode(numcode = 2)
```

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Trp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Thr	CCT	Pro	CAT	His	CGT	Arg
CTC	Thr	CCC	Pro	CAC	His	CGC	Arg
CTA	Thr	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Thr	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Met	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table 1.2: Genetic code number 3: yeast.mitochondrial.

Genetic code 2 : vertebrate.mitochondrial

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Trp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Met	ACA	Thr	AAA	Lys	AGA	Stp
ATG	Met	ACG	Thr	AAG	Lys	AGG	Stp
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

As from **seqinR** 1.0-4, it is possible to export the table of a genetic code into a LaTeX document, for instance table 1.2 and table 1.3 were automatically generated with the following R code:

```
tablecode(numcode = 3, latexfile = "../tables/code3.tex",
    size = "small")
tablecode(numcode = 4, latexfile = "../tables/code4.tex",
    size = "small")
```

The tables were then inserted in the LaTeX file with:

```
\input{../tables/code3.tex}
```

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Trp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table 1.3: Genetic code number 4: protozoan.mitochondrial+mycoplasma.

```
\input{../tables/code4.tex}
```

1.7.6 Data as fast moving targets

In research area, data are not always stable. Consider figure 1 from [33] which is reproduced here in figure 1.1. Data have been updated since then, but we can re-use the same code[8] to update the figure:

```
data <- get.db.growth()
scale <- 1
ltymoore <- 1
date <- data$date
Nucleotides <- data$Nucleotides
Month <- data$Month
plot.default(date, log10(Nucleotides), main = "Update of Fig. 1 from Lobry (2004) LNCS, 3039:679:\nThe e
    xlab = "Year", ylab = "Log10 number of nucleotides", pch = 19,
    cex = scale/2, cex.axis = scale, cex.lab = scale)
abline(lm(log10(Nucleotides) ~ date), lwd = 2)
lm1 <- lm(log(Nucleotides) ~ date)
mu <- lm1$coef[2]
dbt <- log(2)/mu
dbt <- 12 * dbt
x <- mean(date)
y <- mean(log10(Nucleotides))
a <- log10(2)/1.5
b <- y - a * x
lm10 <- lm(log10(Nucleotides) ~ date)
for (i in seq(-10, 10, by = 1)) if (i != 0) abline(coef = c(b +
    i, a), col = "black", lty = ltymoore)
```

[8] This code was copy/pasted from http://pbil.univ-lyon1.fr/members/lobry/repro/lncs04/.

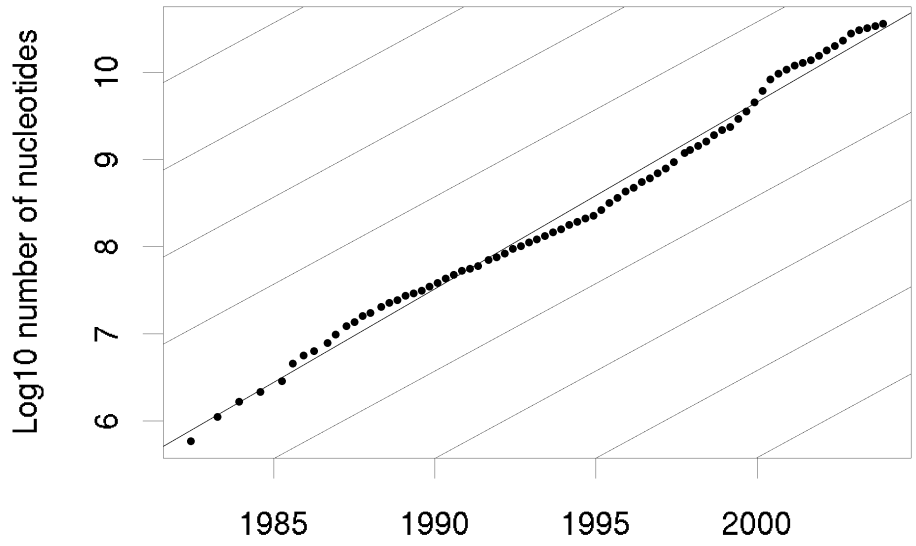
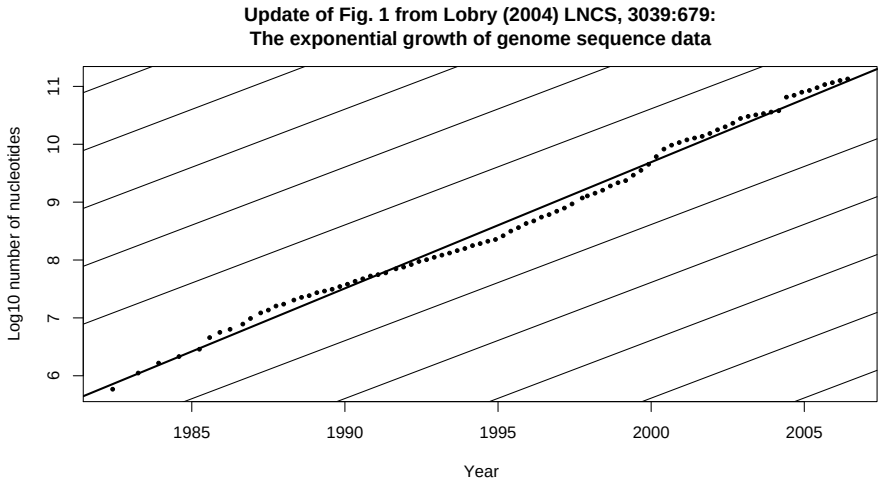


Figure 1.1: Screenshot of figure 1 from [33]. The exponential growth of genomic sequence data mimics Moore's law. The source of data is the december 2003 release note (realnote.txt) from the EMBL database available at `http://www.ebi.ac.uk/`. External lines correspond to what would be expected with a doubling time of 18 months. The central line through points is the best least square fit, corresponding to a doubling time of 16.9 months.



The doubling time is now 16.6 months.

1.7.7 `Sweave()` and `xtable()`

For LaTeX users, it's worth mentioning the fantastic tool contributed by Friedrich Leish [31] called `Sweave()` that allows for the automatic insertion of R outputs

(including graphics) in a LaTeX document. In the same spirit, there is a package called `xtable` [4] to coerce R data into LaTeX tables.

1.8 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

CHAPTER 2

Importing sequences from flat files

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2.1 Importing raw sequence data from FASTA files

2.1.1 FASTA files examples

The FASTA format is very simple and widely used for simple import of biological sequences. It was used originally by the FASTA program [43]. It begins with a single-line description starting with a character >, followed by lines of sequence data of maximum 80 character each. Examples of files in FASTA format are distributed with the **seqinR** package in the `sequences` directory:

```
list.files(path = system.file("sequences", package = "seqinr"),
    pattern = ".fasta")
```

```
 [1] "ATH1_pep_cm_20040228.fasta" "Anouk.fasta"
 [3] "bb.fasta"                   "bordetella.fasta"
 [5] "ct.fasta"                   "ecolicgpe5.fasta"
 [7] "gopher.fasta"               "louse.fasta"
 [9] "malM.fasta"                 "ortho.fasta"
[11] "seqAA.fasta"                "smallAA.fasta"
```

Here is an example of a FASTA file:

```
cat(readLines(system.file("sequences/seqAA.fasta", package = "seqinr")),
    sep = "\n")
```

```
>A06852                  183 residues
MPRLFSYLLGVWLLLSQLPREIPGQSTNDFIKACGRELVRLWVEICGSVSWGRTALSLEE
PQLETGPPAETMPSSITKDAEILKMMLEFVPNLPQELKATLSERQPSLRELQQSASKDSN
LNFEEFKKIILNRQNEAEDKSLLELKNLGLDKHSRKKRLFRMTLSEKCCQVGCIRKDIAR
LC*
```

2.1.2 The function `read.fasta()`

The function `read.fasta()` imports sequences from FASTA files into your workspace.

DNA file example

The example file looks like:

```
dnafile <- system.file("sequences/malM.fasta", package = "seqinr")
cat(readLines(dnafile), sep = "\n")
```

```
>XYLEECOM.MALM  921 bp ACCESSION  E00218, X04477
ATGAAAATGAATAAAAGTCTCATCGTCCTCTGTTTATCAGCAGGGTTACTGGCAAGCGCG
CCTGGAATTAGCCTTGCCGATGTTAACTACGTACCGCAAAACACCAGCGACGCGCCAGCC
ATTCCATCTGCTGCGCTGCAACAACTCACCTGGACACCGGTCGATCAATCTAAAACCCAG
ACCACCCAACTGGCGACCGGCGGCCAACAACTGAACGTTCCCGGCATCAGTGGTCCGGTT
GCTGCGTACAGCGTCCCGGCAAACATTGGCGAACTGACCCTGACGCTGACCAGCGAAGTG
AACAAACAAACCAGCGTTTTTGCGCCGAACGTGCTGATTCTTGATCAGAACATGACCCCA
TCAGCCTTCTTCCCCAGCAGTTATTTCACCTACCAGGAACCAGGCGTGATGAGTGCAGAT
CGGCTGGAAGGCGTTATGCGCCTGACACCGGCGTTGGGGCAGCAAAAACTTTATGTTCTG
GTCTTTACCACGGAAAAAGATCTCCAGCAGACGACCCAACTGCTCGACCCGGCTAAAGCC
TATGCCAAGGGCGTCGGTAACTCGATCCCGGATATCCCCGATCCGGTTGCTCGTCATACC
ACCGATGGCTTACTGAAACTGAAAGTGAAAACGAACTCCAGCTCCAGCGTGTTGGTAGGA
CCCTTATTTGGTTCCTCCGCTCCAGCTCCGGTTACGGTAGGTAACACGGCGGCACCAGCT
GTGGCTGCACCCGCTCCGGCACCGGTGAAGAAAAGCGAGCCGATGCTCAACGACACGGAA
AGTTATTTTAATACCGCGATCAAAAACGCTGTCGCGAAAGGTGATGTTGATAAGGCGTTA
AAACTGCTTGATGAAGCTGAACGCTTGGGATCGACATCTGCCCGTTCCACCTTTATCAGC
AGTGTAAAAGGCAAGGGGTAA
```

With default arguments the output looks like:

```
read.fasta(file = dnafile)
```

```
$XYLEECOM.MALM
  [1] "a" "t" "g" "a" "a" "a" "a" "t" "g" "a" "a" "t" "a" "a" "a" "a" "g" "t"
 [19] "c" "t" "c" "a" "t" "c" "g" "t" "c" "c" "t" "c" "t" "g" "t" "t" "t" "a"
 [37] "t" "c" "a" "g" "c" "a" "g" "g" "g" "t" "t" "a" "c" "t" "g" "g" "c" "a"
 [55] "a" "g" "c" "g" "c" "g" "c" "c" "t" "g" "g" "a" "a" "t" "t" "a" "g" "c"
 [73] "c" "t" "t" "g" "c" "c" "g" "a" "t" "g" "t" "t" "a" "a" "c" "t" "a" "c"
 [91] "g" "t" "a" "c" "c" "g" "c" "a" "a" "a" "a" "c" "a" "c" "c" "a" "g" "c"
[109] "g" "a" "c" "g" "c" "g" "c" "c" "a" "g" "c" "c" "a" "t" "t" "c" "c" "a"
[127] "t" "c" "t" "g" "c" "t" "g" "c" "g" "c" "t" "g" "c" "a" "a" "c" "a" "a"
[145] "c" "t" "c" "a" "c" "c" "t" "g" "g" "a" "c" "a" "c" "c" "g" "g" "t" "c"
[163] "g" "a" "t" "c" "a" "a" "t" "c" "t" "a" "a" "a" "a" "c" "c" "c" "a" "g"
[181] "a" "c" "c" "a" "c" "c" "c" "a" "a" "c" "t" "g" "g" "c" "g" "a" "c" "c"
[199] "g" "g" "c" "g" "g" "c" "c" "a" "a" "c" "a" "a" "c" "t" "g" "a" "a" "c"
[217] "g" "t" "t" "c" "c" "c" "g" "g" "c" "a" "t" "c" "a" "g" "t" "g" "g" "t"
[235] "c" "c" "g" "g" "t" "t" "g" "c" "t" "g" "c" "g" "t" "a" "c" "a" "g" "c"
[253] "g" "t" "c" "c" "c" "g" "g" "c" "a" "a" "a" "c" "a" "t" "t" "g" "g" "c"
[271] "g" "a" "a" "c" "t" "g" "a" "c" "c" "c" "t" "g" "a" "c" "g" "c" "t" "g"
[289] "a" "c" "c" "a" "g" "c" "g" "a" "a" "g" "t" "g" "a" "a" "c" "a" "a" "a"
[307] "c" "a" "a" "a" "c" "c" "a" "g" "c" "g" "t" "t" "t" "t" "t" "g" "c" "g"
[325] "c" "c" "g" "a" "a" "c" "g" "t" "g" "c" "t" "g" "a" "t" "t" "c" "t" "t"
[343] "g" "a" "t" "c" "a" "g" "a" "a" "c" "a" "t" "g" "a" "c" "c" "c" "c" "a"
[361] "t" "c" "a" "g" "c" "c" "t" "t" "c" "t" "t" "c" "c" "c" "c" "a" "g" "c"
[379] "a" "g" "t" "t" "a" "t" "t" "t" "c" "a" "c" "c" "t" "a" "c" "c" "a" "g"
[397] "g" "a" "a" "c" "c" "a" "g" "g" "c" "g" "t" "g" "a" "t" "g" "a" "g" "t"
[415] "g" "c" "a" "g" "a" "t" "c" "g" "g" "c" "t" "g" "g" "a" "a" "g" "g" "c"
[433] "g" "t" "t" "a" "t" "g" "c" "g" "c" "c" "t" "g" "a" "c" "a" "c" "c" "g"
[451] "g" "c" "g" "t" "t" "g" "g" "g" "g" "c" "a" "g" "c" "a" "a" "a" "a" "a"
```

```
[469] "c" "t" "t" "t" "a" "t" "g" "t" "t" "c" "t" "g" "g" "t" "c" "t" "t" "t"
[487] "a" "c" "c" "a" "c" "g" "g" "a" "a" "a" "a" "a" "g" "a" "t" "c" "t" "c"
[505] "c" "a" "g" "c" "a" "g" "a" "c" "g" "a" "c" "c" "c" "a" "a" "c" "t" "g"
[523] "c" "t" "c" "g" "a" "c" "c" "c" "g" "g" "c" "t" "a" "a" "a" "g" "c" "c"
[541] "t" "a" "t" "g" "c" "c" "a" "a" "g" "g" "g" "c" "g" "t" "c" "g" "g" "t"
[559] "a" "a" "c" "t" "c" "g" "a" "t" "c" "c" "c" "g" "g" "a" "t" "a" "t" "c"
[577] "c" "c" "c" "g" "a" "t" "c" "c" "g" "g" "t" "t" "g" "c" "t" "c" "g" "t"
[595] "c" "a" "t" "a" "c" "c" "a" "c" "c" "g" "a" "t" "g" "g" "c" "t" "t" "a"
[613] "c" "t" "g" "a" "a" "a" "c" "t" "g" "a" "a" "a" "g" "t" "g" "a" "a" "a"
[631] "a" "c" "g" "a" "a" "c" "t" "c" "c" "a" "g" "c" "t" "c" "c" "a" "g" "c"
[649] "g" "t" "g" "t" "t" "g" "g" "t" "a" "g" "g" "a" "c" "c" "c" "t" "t" "a"
[667] "t" "t" "t" "g" "g" "t" "t" "c" "c" "t" "c" "c" "g" "c" "t" "c" "c" "a"
[685] "g" "c" "t" "c" "c" "g" "g" "t" "t" "a" "c" "g" "g" "t" "a" "g" "g" "t"
[703] "a" "a" "c" "a" "c" "g" "g" "c" "g" "g" "c" "a" "c" "c" "a" "g" "c" "t"
[721] "g" "t" "g" "g" "c" "t" "g" "c" "a" "c" "c" "c" "g" "c" "t" "c" "c" "g"
[739] "g" "c" "a" "c" "c" "g" "g" "t" "g" "a" "a" "g" "a" "a" "a" "a" "g" "c"
[757] "g" "a" "g" "c" "c" "g" "a" "t" "g" "c" "t" "c" "a" "a" "c" "g" "a" "c"
[775] "a" "c" "g" "g" "a" "a" "a" "g" "t" "t" "a" "t" "t" "t" "t" "a" "a" "t"
[793] "a" "c" "c" "g" "c" "g" "a" "t" "c" "a" "a" "a" "a" "a" "c" "g" "c" "t"
[811] "g" "t" "c" "g" "c" "g" "a" "a" "a" "g" "g" "t" "g" "a" "t" "g" "t" "t"
[829] "g" "a" "t" "a" "a" "g" "g" "c" "g" "t" "t" "a" "a" "a" "a" "c" "t" "g"
[847] "c" "t" "t" "g" "a" "t" "g" "a" "a" "g" "c" "t" "g" "a" "a" "c" "g" "c"
[865] "t" "t" "g" "g" "g" "a" "t" "c" "g" "a" "c" "a" "t" "c" "t" "g" "c" "c"
[883] "c" "g" "t" "t" "c" "c" "a" "c" "c" "t" "t" "t" "a" "t" "c" "a" "g" "c"
[901] "a" "g" "t" "g" "t" "a" "a" "a" "a" "g" "g" "c" "a" "a" "g" "g" "g" "g"
[919] "t" "a" "a"
attr(,"name")
[1] "XYLEECOM.MALM"
attr(,"Annot")
[1] ">XYLEECOM.MALM  921 bp ACCESSION  E00218, X04477"
attr(,"class")
[1] "SeqFastadna"
```

As from **seqinR** 1.0-5 the automatic conversion of sequences into vector of single characters can be neutralized, for instance:

```
read.fasta(file = dnafile, as.string = TRUE)
```

```
$XYLEECOM.MALM
[1] "atgaaaatgaataaaagtctcatcgtcctctgtttatcagcagggttactggcaagcgcgcctggaattagccttgccgatgttaactacgtaccgcaaaacaccagcgacg
attr(,"name")
[1] "XYLEECOM.MALM"
attr(,"Annot")
[1] ">XYLEECOM.MALM  921 bp ACCESSION  E00218, X04477"
attr(,"class")
[1] "SeqFastadna"
```

Forcing to lower case letters can be disabled this way:

```
read.fasta(file = dnafile, as.string = TRUE, forceDNAtolower = FALSE)
```

```
$XYLEECOM.MALM
[1] "ATGAAAATGAATAAAAGTCTCATCGTCCTCTGTTTATCAGCAGGGTTACTGGCAAGCGCGCCTGGAATTAGCCTTGCCGATGTTAACTACGTACCGCAAAACACCAGCGACG
attr(,"name")
[1] "XYLEECOM.MALM"
attr(,"Annot")
[1] ">XYLEECOM.MALM  921 bp ACCESSION  E00218, X04477"
attr(,"class")
[1] "SeqFastadna"
```

Protein file example

The example file looks like:

```
aafile <- system.file("sequences/seqAA.fasta", package = "seqinr")
cat(readLines(aafile), sep = "\n")
```

```
>A06852                  183 residues
MPRLFSYLLGVWLLLSQLPREIPGQSTNDFIKACGRELVRLWVEICGSVSWGRTALSLEE
PQLETGPPAETMPSSITKDAEILKMMLEFVPNLPQELKATLSERQPSLRELQQSASKDSN
LNFEEFKKIILNRQNEAEDKSLLELKNLGLDKHSRKKRLFRMTLSEKCCQVGCIRKDIAR
LC*
```

Read the protein sequence file, looks like:

```
read.fasta(aafile, seqtype = "AA")
```

```
$A06852
  [1] "M" "P" "R" "L" "F" "S" "Y" "L" "L" "G" "V" "W" "L" "L" "L" "S" "Q" "L"
 [19] "P" "R" "E" "I" "P" "G" "Q" "S" "T" "N" "D" "F" "I" "K" "A" "C" "G" "R"
 [37] "E" "L" "V" "R" "L" "W" "V" "E" "I" "C" "G" "S" "V" "S" "W" "G" "R" "T"
 [55] "A" "L" "S" "L" "E" "E" "P" "Q" "L" "E" "T" "G" "P" "P" "A" "E" "T" "M"
 [73] "P" "S" "S" "I" "T" "K" "D" "A" "E" "I" "L" "K" "M" "M" "L" "E" "F" "V"
 [91] "P" "N" "L" "P" "Q" "E" "L" "K" "A" "T" "L" "S" "E" "R" "Q" "P" "S" "L"
[109] "R" "E" "L" "Q" "Q" "S" "A" "S" "K" "D" "S" "N" "L" "N" "F" "E" "E" "F"
[127] "K" "K" "I" "I" "L" "N" "R" "Q" "N" "E" "A" "E" "D" "K" "S" "L" "L" "E"
[145] "L" "K" "N" "L" "G" "L" "D" "K" "H" "S" "R" "K" "K" "R" "L" "F" "R" "M"
[163] "T" "L" "S" "E" "K" "C" "C" "Q" "V" "G" "C" "I" "R" "K" "D" "I" "A" "R"
[181] "L" "C" "*"
attr(,"name")
[1] "A06852"
attr(,"Annot")
[1] ">A06852                  183 residues"
attr(,"class")
[1] "SeqFastaAA"
```

The same, but as string and without attributes setting, looks like:

```
read.fasta(aafile, seqtype = "AA", as.string = TRUE, set.attributes = FALSE)
```

```
$A06852
[1] "MPRLFSYLLGVWLLLSQLPREIPGQSTNDFIKACGRELVRLWVEICGSVSWGRTALSLEEPQLETGPPAETMPSSITKDAEILKMMLEFVPNLPQELKAT
```

2.1.3 The function `write.fasta()`

This function writes sequences to a file in FASTA format. Read 3 coding sequences sequences from a FASTA file:

```
ortho <- read.fasta(file = system.file("sequences/ortho.fasta",
    package = "seqinr"))
length(ortho)
```

```
[1] 3
```

```
ortho[[1]][1:12]
```

```
 [1] "a" "t" "g" "g" "c" "t" "c" "a" "g" "c" "g" "g"
```

Select only third codon positions:

```
ortho3 <- lapply(ortho, function(x) x[seq(from = 3, to = length(x),
    by = 3)])
ortho3[[1]][1:4]
```

```
[1] "g" "t" "g" "g"
```

Write the modified sequences to a file:

```
tmpf <- tempfile()
write.fasta(sequences = ortho3, names = names(ortho3), nbchar = 80,
    file.out = tmpf)
```

Read them again from the same file and check that sequences are preserved:

```
ortho3bis <- read.fasta(tmpf, set.attributes = FALSE)
identical(ortho3bis, ortho3)
```

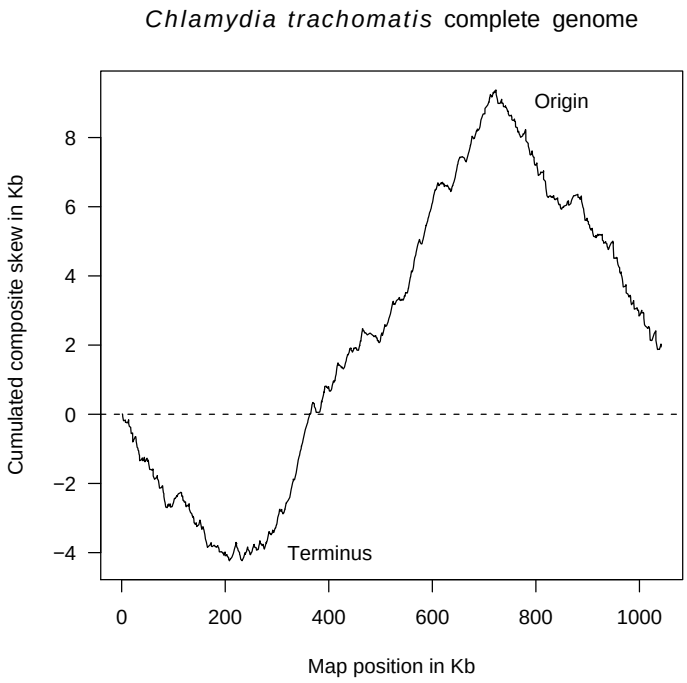
```
[1] TRUE
```

2.1.4 Big room examples

Oriloc example (*Chlamydia trachomatis* complete genome)

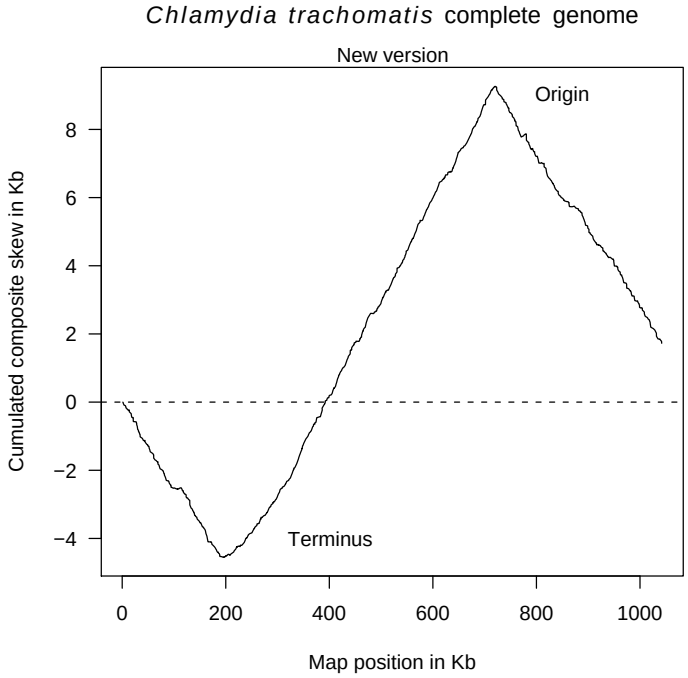
A more consequent example is given in the fasta file `ct.fasta` which contains the complete genome of *Chlamydia trachomatis* that was used in [7]. You should be able to reproduce figure 1b from this paper with the following code:

```
out <- oriloc(seq.fasta = system.file("sequences/ct.fasta",
    package = "seqinr"), g2.coord = system.file("sequences/ct.coord",
    package = "seqinr"), oldoriloc = TRUE)
plot(out$st, out$sk/1000, type = "l", xlab = "Map position in Kb",
    ylab = "Cumulated composite skew in Kb", main = expression(italic(Chlamydia ~
        ~trachomatis) ~ ~complete ~ ~genome), las = 1)
abline(h = 0, lty = 2)
text(400, -4, "Terminus")
text(850, 9, "Origin")
```



Note that the algorithm has been improved since then and that it's more advisable to use the default option `oldoriloc = FALSE` if you are interested in the prediction of origins and terminus of replication from base composition biases (more on this at `http://pbil.univ-lyon1.fr/software/oriloc.html`). See also [38] for a recent review on this topic.

```
out <- oriloc(seq.fasta = system.file("sequences/ct.fasta",
    package = "seqinr"), g2.coord = system.file("sequences/ct.coord",
    package = "seqinr"))
plot(out$st, out$sk/1000, type = "l", xlab = "Map position in Kb",
    ylab = "Cumulated composite skew in Kb", main = expression(italic(Chlamydia ~
        ~trachomatis) ~ ~complete ~ ~genome), las = 1)
mtext("New version")
abline(h = 0, lty = 2)
text(400, -4, "Terminus")
text(850, 9, "Origin")
```




Arabidobpsis thaliana. Source: wikipedia.

Example with 21,161 proteins from *Arabidobpsis thaliana*

As from **seqinR** 1.0-5 the automatic conversion of sequences into vector of single characters and the automatic attribute settings can be neutralized, for instance :

```
smallAA <- system.file("sequences/smallAA.fasta", package = "seqinr")
read.fasta(smallAA, seqtype = "AA", as.string = TRUE, set.attributes = FALSE)
```

```
$smallAA
[1] "SEQINRSEQINRSEQINRSEQINR*"
```

This is interesting to save time and space when reading large FASTA files. Let's give a practical example. In their paper [17], Matthew Hannah, Arnd Heyer and Dirk Hincha were working on *Arabidobpsis thaliana* genes in order to detect those involved in cold acclimation. They were interested by the detection of proteins called hydrophilins, that had a mean hydrophilicity of over 1 and glycine content of over 0.08 [9], because they are though to be important for freezing tolerance. The starting point was a FASTA file called `ATH1_pep_cm_20040228` downloaded from the Arabidopsis Information Ressource (TAIR at `http://www.arabidopsis.org/`) which contains the sequences of 21,161 proteins.

```
athfile <- system.file("sequences/ATH1_pep_cm_20040228.fasta",
    package = "seqinr")
system.time(ath <- read.fasta(athfile, seqtype = "AA", as.string = TRUE,
    set.attributes = FALSE))
```

```
[1] 28.222  0.461 29.937  0.000  0.000
```

It's about one minute here to read 21,161 protein sequences. We save them in XDR binary format to read them faster later at will:

```
save(ath, file = "ath.RData")

system.time(load("ath.RData"))
```

```
[1] 0.655 0.048 1.009 0.000 0.000
```

Now it's about one second to load the whole data set thanks to the XDR format. The object size is about 15 Mo in RAM, that is something very close to the flat file size on disk:

```
object.size(ath)/2^20
```

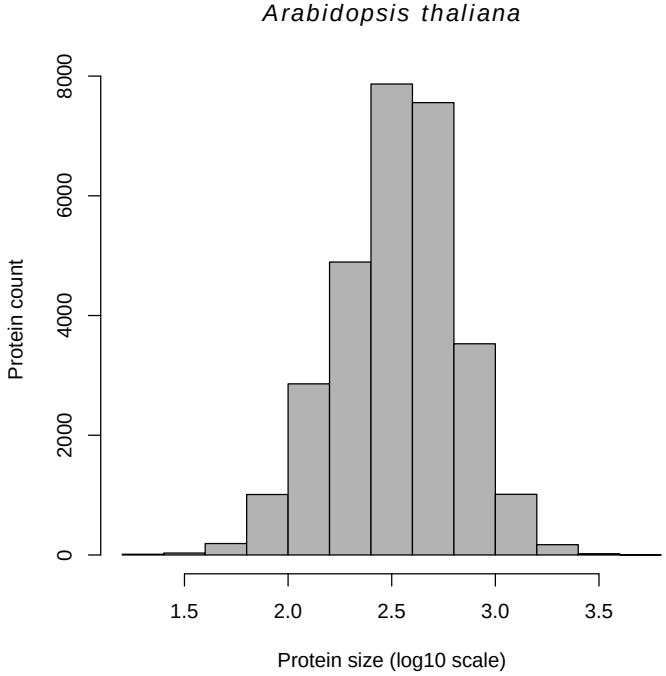
```
[1] 14.94021
```

```
file.info(athfile)$size/2^20
```

```
[1] 15.89863
```

Using strings for sequence storage is very comfortable when there is an efficient function to compute what you want. For instance, suppose that you are interested by the distribution of protein size in *Arabidopsis thaliana*. There is an efficient vectorized function called `nchar()` that will do the job, we just have to remove one unit because of the stop codon which is translated as a star (*) in this data set. This is a simple and direct task under R:

```
nres <- nchar(ath) - 1
hist(log10(nres), col = grey(0.7), xlab = "Protein size (log10 scale)",
    ylab = "Protein count", main = expression(italic(Arabidopsis ~
        ~thaliana)))
```



However, sometimes it is more convenient to work with the single character vector representation of sequences. For instance, to count the number of glycine (G), we first play with one sequence, let's take the smallest one in the data set:

```
which.min(nres)
```

```
[1] 9523
```

```
ath[[9523]]
```

```
[1] "MAGSQREKLKPRTKGSTRC*"
```

```
s2c(ath[[9523]])
```

```
 [1] "M" "A" "G" "S" "Q" "R" "E" "K" "L" "K" "P" "R" "T" "K" "G" "S" "T" "R"
[19] "C" "*"
```

```
s2c(ath[[9523]]) == "G"
```

```
 [1] FALSE FALSE  TRUE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE FALSE
[13] FALSE FALSE  TRUE FALSE FALSE FALSE FALSE FALSE
```

```
sum(s2c(ath[[9523]]) == "G")
```

```
[1] 2
```

We can now easily define a vectorised function to count the number of glycine:

```
ngly <- function(data) {
    res <- sapply(data, function(x) sum(s2c(x) == "G"))
    names(res) <- NULL
    return(res)
}
```

Now we can use `ngly()` in the same way that `nchar()` so that computing glycine frequencies is very simple:

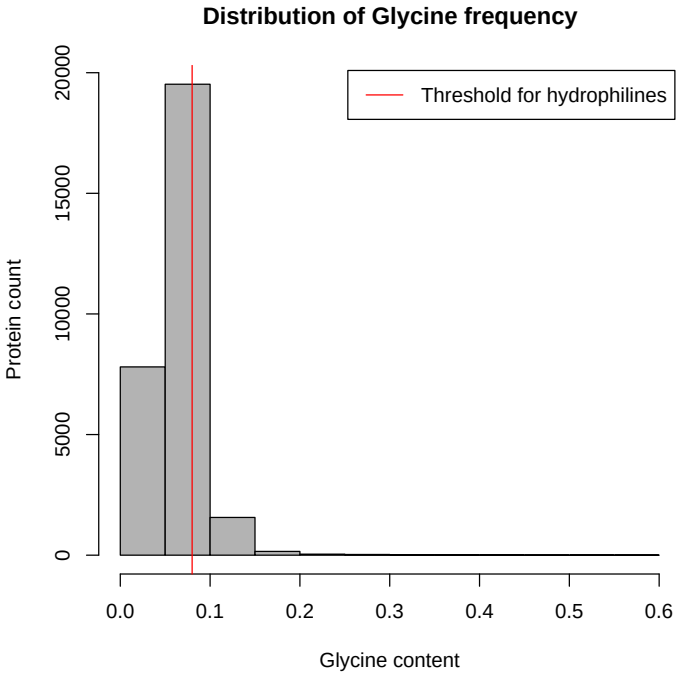
```
ngly(ath[1:10])
```

```
 [1]  25   5  29 128   8  27  27  26  21  18
```

```
fgly <- ngly(ath)/nres
```

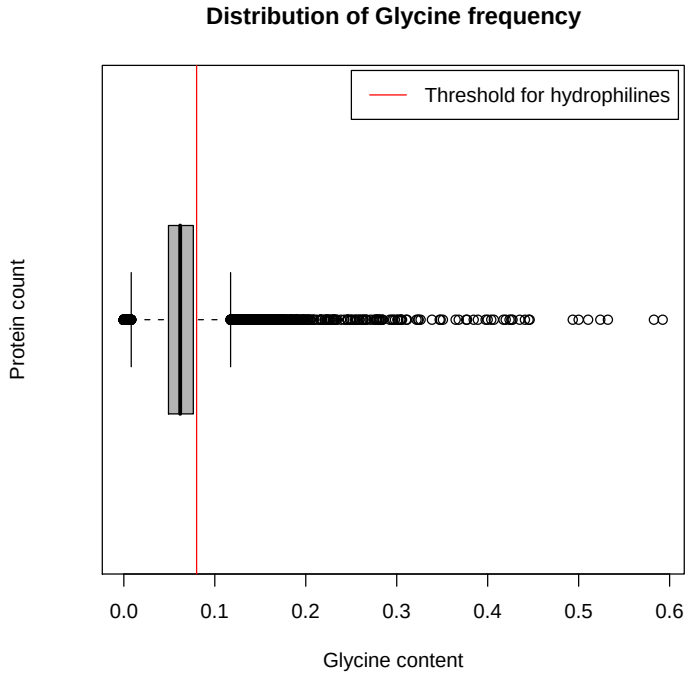
And we can have a look at the distribution:

```
hist(fgly, col = grey(0.7), main = "Distribution of Glycine frequency",
    xlab = "Glycine content", ylab = "Protein count")
abline(v = 0.08, col = "red")
legend("topright", inset = 0.01, lty = 1, col = "red", legend = "Threshold for hydrophilines")
```



Let's use a boxplot instead:

```
boxplot(fgly, horizontal = TRUE, col = grey(0.7), main = "Distribution of Glycine frequency",
    xlab = "Glycine content", ylab = "Protein count")
abline(v = 0.08, col = "red")
legend("topright", inset = 0.01, lty = 1, col = "red", legend = "Threshold for hydrophilines")
```



The threshold value for the glycine content in hydrophilines is therefore very close to the third quartile of the distribution:

```
summary(fgly)
```

```
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
0.00000 0.04907 0.06195 0.06475 0.07639 0.59240
```

We want now to compute something relatively more complex, we want the Kyte and Doolittle[29] hydropathy score of our proteins (aka GRAVY score). This is basically a linear form on amino acid frequencies:

$$s = \sum_{i=1}^{20} \alpha_i f_i$$

where α_i is the coefficient for amino acid number i and f_i the relative frequency of amino acid number i. The coefficients α_i are given in the `KD` component of the data set `EXP`:

```
data(EXP)
EXP$KD
```

```
 [1] -3.9 -3.5 -3.9 -3.5 -0.7 -0.7 -0.7 -0.7 -4.5 -0.8 -4.5 -0.8  4.5  4.5
[15]  1.9  4.5 -3.5 -3.2 -3.5 -3.2 -1.6 -1.6 -1.6 -1.6 -4.5 -4.5 -4.5 -4.5
[29]  3.8  3.8  3.8  3.8 -3.5 -3.5 -3.5 -3.5  1.8  1.8  1.8  1.8 -0.4 -0.4
[43] -0.4 -0.4  4.2  4.2  4.2  4.2  0.0 -1.3  0.0 -1.3 -0.8 -0.8 -0.8 -0.8
[57]  0.0  2.5 -0.9  2.5  3.8  2.8  3.8  2.8
```

This is for codons in lexical order, that is:

```
words()
```

```
 [1] "aaa" "aac" "aag" "aat" "aca" "acc" "acg" "act" "aga" "agc" "agg" "agt"
[13] "ata" "atc" "atg" "att" "caa" "cac" "cag" "cat" "cca" "ccc" "ccg" "cct"
[25] "cga" "cgc" "cgg" "cgt" "cta" "ctc" "ctg" "ctt" "gaa" "gac" "gag" "gat"
[37] "gca" "gcc" "gcg" "gct" "gga" "ggc" "ggg" "ggt" "gta" "gtc" "gtg" "gtt"
[49] "taa" "tac" "tag" "tat" "tca" "tcc" "tcg" "tct" "tga" "tgc" "tgg" "tgt"
[61] "tta" "ttc" "ttg" "ttt"
```

But since we are working with protein sequences here we name the coefficient according to their amino acid :

```
names(EXP$KD) <- sapply(words(), function(x) translate(s2c(x)))
```

We just need one value per amino acid, we sort them in the lexical order, and we reverse the scale so as to have positive values for hydrophilic proteins as in [17] :

```
kdc <- EXP$KD[unique(names(EXP$KD))]
kdc <- -kdc[order(names(kdc))]
kdc
```

```
  *   A   C   D   E   F   G   H   I   K   L   M   N   P   Q
0.0 1.8 2.5 3.5 3.5 2.8 0.4 3.2 4.5 3.9 3.8 1.9 3.5 1.6 3.5
  R   S   T   V   W   Y
4.5 0.8 0.7 4.2 0.9 1.3
```

Now that we have the vector of coefficient α_i, we need the amino acid relative frequencies f_i, let's play with one protein first:

```
ath[[9523]]
```

```
[1] "MAGSQREKLKPRTKGSTRC*"
```

```
s2c(ath[[9523]])
```

```
 [1] "M" "A" "G" "S" "Q" "R" "E" "K" "L" "K" "P" "R" "T" "K" "G" "S" "T" "R"
[19] "C" "*"
```

```
table(s2c(ath[[9523]]))
```

```
* A C E G K L M P Q R S T
1 1 1 1 2 3 1 1 1 1 3 2 2
```

```
table(factor(s2c(ath[[9523]]), levels = names(kdc)))
```

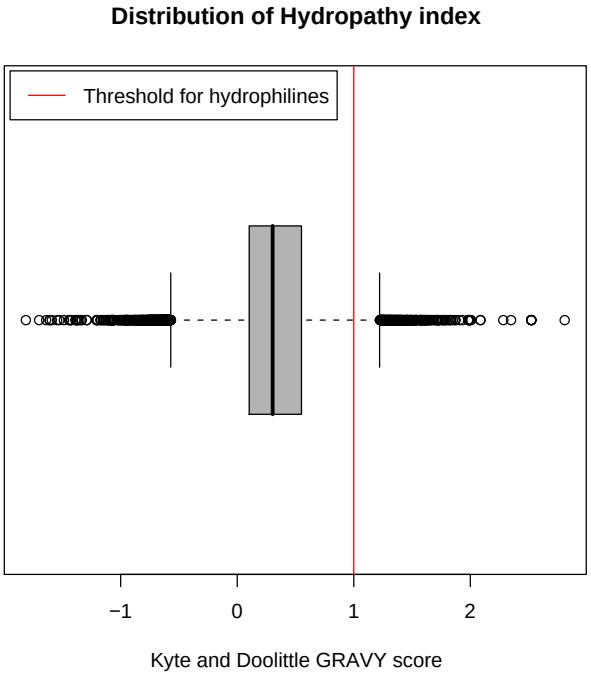
```
* A C D E F G H I K L M N P Q R S T V W Y
1 1 1 0 1 0 2 0 0 3 1 1 0 1 1 3 2 2 0 0 0
```

Now that we know how to count amino acids it's relatively easy thanks to R's matrix operator `%*%` to define a vectorised function to compute a linear form on amino acid frequencies:

```
linform <- function(data, coef) {
    f <- function(x) {
        aaseq <- s2c(x)
        freq <- table(factor(aaseq, levels = names(coef)))/length(aaseq)
        return(coef %*% freq)
    }
    res <- sapply(data, f)
    names(res) <- NULL
    return(res)
}
kdath <- linform(ath, kdc)
```

Let's have a look at the distribution:

```
boxplot(kdath, horizontal = TRUE, col = grey(0.7), main = "Distribution of Hydropathy index",
    xlab = "Kyte and Doolittle GRAVY score")
abline(v = 1, col = "red")
legend("topleft", inset = 0.01, lty = 1, col = "red", legend = "Threshold for hydrophilines")
```



The threshold is therefore much more stringent here than the previous one on glycine content. Let's define a vector of logicals to select the hydrophilines:

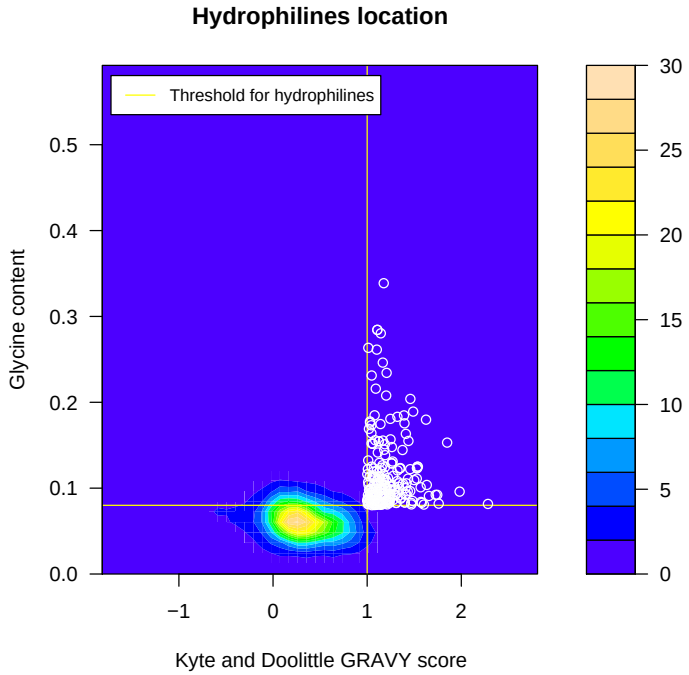
```
hydrophilines <- fgly > 0.08 & kdath > 1
names(ath)[hydrophilines]
```

```
  [1] "At1g02840.1" "At1g02840.2" "At1g02840.3" "At1g03320.1" "At1g03820.1"
  [6] "At1g04450.1" "At1g12810.1" "At1g13020.1" "At1g13290.1" "At1g13890.1"
 [11] "At1g15270.1" "At1g15280.1" "At1g15280.2" "At1g15400.1" "At1g15400.2"
 [16] "At1g20220.1" "At1g21520.1" "At1g23530.1" "At1g23860.1" "At1g23860.2"
 [21] "At1g26210.1" "At1g27650.1" "At1g31750.1" "At1g42430.1" "At1g44478.1"
 [26] "At1g47970.1" "At1g48920.1" "At1g49680.1" "At1g50300.1" "At1g51510.1"
 [31] "At1g52300.1" "At1g52690.1" "At1g52690.2" "At1g52710.1" "At1g53160.1"
 [36] "At1g53160.2" "At1g53260.1" "At1g53645.1" "At1g54410.1" "At1g54926.1"
 [41] "At1g55310.1" "At1g56085.1" "At1g60650.1" "At1g60650.2" "At1g62045.1"
 [46] "At1g63310.1" "At1g64360.1" "At1g64370.1" "At1g67325.1" "At1g67870.1"
 [51] "At1g72050.1" "At1g72080.1" "At1g73350.1" "At1g75560.1" "At1g76010.1"
 [56] "At1g77030.1" "At1g77765.1" "At1g80000.1" "At1g80000.2" "At2g02160.1"
 [61] "At2g03440.1" "At2g04380.1" "At2g04870.1" "At2g07675.1" "At2g11910.1"
 [66] "At2g11910.2" "At2g13125.1" "At2g17950.1" "At2g19750.1" "At2g20430.1"
 [71] "At2g20760.1" "At2g21490.1" "At2g22080.1" "At2g22100.1" "At2g22820.1"
 [76] "At2g23110.1" "At2g24590.1" "At2g25990.1" "At2g27100.1" "At2g32710.1"
 [81] "At2g32710.2" "At2g33460.1" "At2g33690.1" "At2g34310.1" "At2g34310.2"
 [86] "At2g34310.3" "At2g35230.2" "At2g37340.1" "At2g37340.2" "At2g37340.3"
 [91] "At2g39855.2" "At2g40040.1" "At2g40080.1" "At2g40170.1" "At2g41730.1"
 [96] "At2g42190.1" "At2g43370.1" "At2g43970.2" "At2g44710.1" "At2g44820.1"
[101] "At2g44820.2" "At2g46270.2" "At3g02480.1" "At3g05220.1" "At3g05220.2"
[106] "At3g07195.1" "At3g10020.1" "At3g13570.1" "At3g13780.1" "At3g15270.1"
[111] "At3g16040.1" "At3g16080.1" "At3g17160.1" "At3g17190.1" "At3g20910.1"
```

```
[116] "At3g21270.1" "At3g21290.1" "At3g22660.1" "At3g23140.1" "At3g23380.1"
[121] "At3g23390.1" "At3g24100.1" "At3g24740.1" "At3g25590.1" "At3g26400.1"
[126] "At3g26420.1" "At3g29075.1" "At3g29470.1" "At3g29600.1" "At3g47120.1"
[131] "At3g48180.1" "At3g49430.1" "At3g50320.1" "At3g50610.1" "At3g50670.1"
[136] "At3g50690.1" "At3g50970.1" "At3g50980.1" "At3g51810.1" "At3g51940.1"
[141] "At3g53500.1" "At3g53500.2" "At3g55460.1" "At3g56720.1" "At3g57930.1"
[146] "At3g58070.1" "At3g60230.1" "At3g62790.1" "At3g63100.1" "At3g63400.1"
[151] "At3g63400.2" "At4g02425.1" "At4g02430.2" "At4g07940.1" "At4g09840.1"
[156] "At4g09850.1" "At4g09980.1" "At4g12610.1" "At4g12670.1" "At4g13560.1"
[161] "At4g13615.1" "At4g14020.1" "At4g14320.1" "At4g16830.1" "At4g17520.1"
[166] "At4g22740.1" "At4g22740.2" "At4g24500.1" "At4g24500.2" "At4g24680.1"
[171] "At4g25340.1" "At4g25500.1" "At4g25500.2" "At4g26640.1" "At4g26640.2"
[176] "At4g28870.1" "At4g28990.1" "At4g29390.1" "At4g31580.1" "At4g36600.1"
[181] "At4g36730.1" "At4g36730.2" "At4g38710.1" "At5g02020.1" "At5g03990.1"
[186] "At5g04290.1" "At5g11260.1" "At5g11970.1" "At5g12840.1" "At5g12840.2"
[191] "At5g12840.3" "At5g12840.4" "At5g16470.1" "At5g17650.1" "At5g19960.1"
[196] "At5g22040.1" "At5g22650.1" "At5g22650.2" "At5g26890.1" "At5g27690.1"
[201] "At5g28610.1" "At5g28630.1" "At5g44200.1" "At5g47210.1" "At5g50830.1"
[206] "At5g51840.1" "At5g52200.1" "At5g52310.1" "At5g52530.1" "At5g52530.2"
[211] "At5g53820.1" "At5g55430.1" "At5g55640.1" "At5g55670.1" "At5g56670.1"
[216] "At5g58040.1" "At5g58470.1" "At5g58470.2" "At5g59080.1" "At5g59950.1"
[221] "At5g59950.3" "At5g60800.1" "At5g60880.1" "At5g61890.1" "At5g64130.1"
[226] "At5g66400.1" "At5g66780.1" "At5g67190.1" "At5g67490.1" "AtMg00880"
```

Check with a simple graph that there is no mistake here:

```
library(MASS)
dst <- kde2d(kdath, fgly, n = 50)
filled.contour(x = dst, color.palette = topo.colors, plot.axes = {
    axis(1)
    axis(2)
    title(xlab = "Kyte and Doolittle GRAVY score", ylab = "Glycine content",
        main = "Hydrophilines location")
    abline(v = 1, col = "yellow")
    abline(h = 0.08, col = "yellow")
    points(kdath[hydrophilines], fgly[hydrophilines], col = "white")
    legend("topleft", inset = 0.02, lty = 1, col = "yellow",
        bg = "white", legend = "Threshold for hydrophilines",
        cex = 0.8)
})
```



Everything seems to be OK, we can save the results in a data frame:

```
athres <- data.frame(list(name = names(ath), KD = kdath, Gly = fgly))
head(athres)
```

```
         name         KD        Gly
1 At1g01010.1  0.7297674 0.05827506
2 At1g01020.1 -0.1674419 0.03906250
3 At1g01030.1  0.8136490 0.08100559
4 At1g01040.1  0.4159686 0.06705081
5 At1g01050.1  0.4460094 0.03773585
6 At1g01060.1  0.7444272 0.04186047
```

We want to check now that the results are consistent with those reported previously. The following table is extracted from the file `pgen.0010026.st003.xls` provided as the supplementary material table S3 in [17] and available at `http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1189076&blobname=pgen.0010026.st003.xls`. Only the protein names, the hydrophilicity and the glycine content were extracted:

```
hannah <- read.table(system.file("sequences/hannah.txt", package = "seqinr"),
    sep = "\t", header = TRUE)
head(hannah)
```

```
       AGI Hydrophilicity Glycine
1 At2g19570          -0.10    0.07
2 At2g45290          -0.25    0.09
3 At4g29570          -0.05    0.07
4 At4g29580          -0.10    0.06
5 At4g29600          -0.14    0.06
6 At5g28050          -0.11    0.08
```

The protein names are not exactly the same because they have no extension. As explained in [17], when multiple gene models were predicted only the first was one used. Then:

```
hannah$AGI <- paste(hannah$AGI, "1", sep = ".")
head(hannah)
```

```
          AGI Hydrophilicity Glycine
1 At2g19570.1          -0.10    0.07
2 At2g45290.1          -0.25    0.09
3 At4g29570.1          -0.05    0.07
4 At4g29580.1          -0.10    0.06
5 At4g29600.1          -0.14    0.06
6 At5g28050.1          -0.11    0.08
```

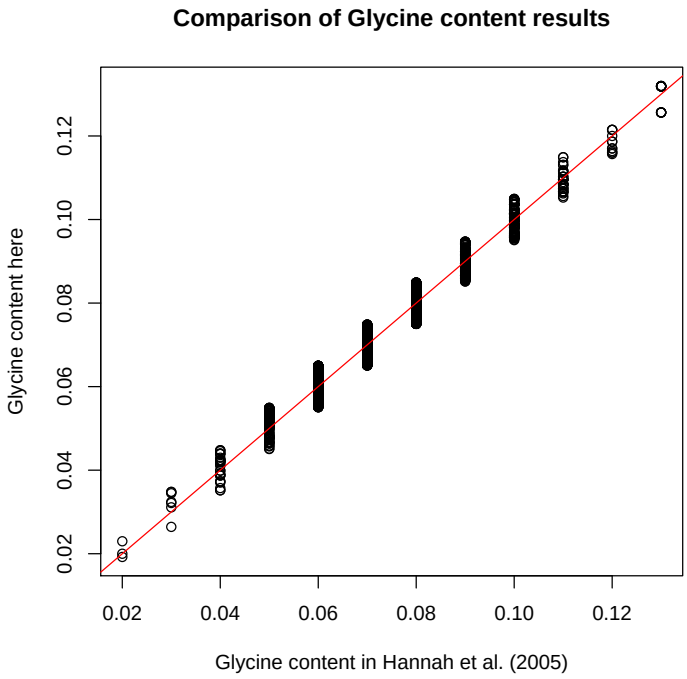
We join now the two data frames thanks to their common key:

```
join <- merge(hannah, athres, by.x = "AGI", by.y = "name")
head(join)
```

```
          AGI Hydrophilicity Glycine         KD        Gly
1 At1g01120.1          -0.10    0.06 0.10699433 0.05871212
2 At1g01390.1           0.02    0.06 0.00914761 0.06458333
3 At1g01390.1           0.02    0.06 0.00914761 0.06458333
4 At1g01420.1          -0.05    0.07 0.06203320 0.07276507
5 At1g01420.1          -0.05    0.07 0.06203320 0.07276507
6 At1g01480.1          -0.20    0.07 0.20080483 0.06653226
```

Let's compare the glycine content :

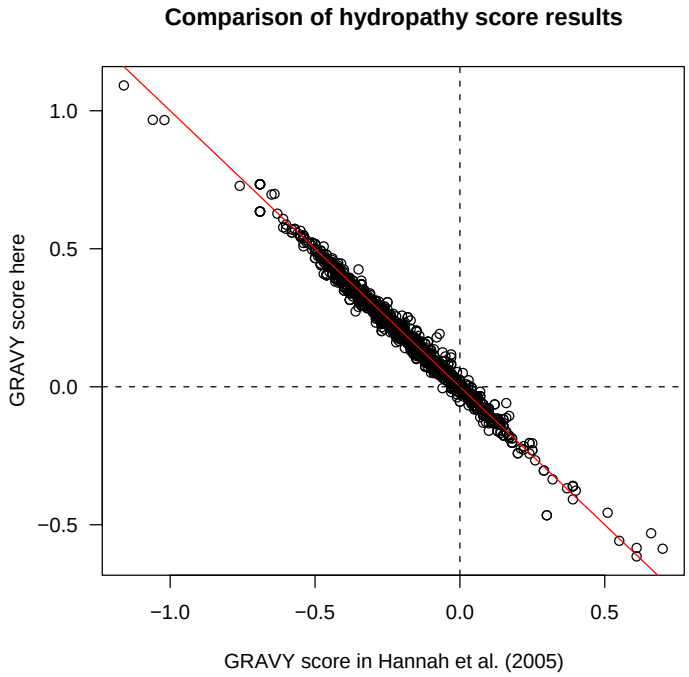
```
plot(join$Glycine, join$Gly, xlab = "Glycine content in Hannah et al. (2005)",
    ylab = "Glycine content here", main = "Comparison of Glycine content results")
abline(c(0, 1), col = "red")
```



The results are consistent, we have just lost some resolution because there are only two figures after the decimal point in the Excel[1] file. Let's have a look at the GRAVY score now:

[1] this software is a real **pain** for the reproducibility of results. This is well documented, see http://www.burns-stat.com/pages/Tutor/spreadsheet_addiction.html and references therein.

```
plot(join$Hydrophilicity, join$KD, xlab = "GRAVY score in Hannah et al. (2005)",
    ylab = "GRAVY score here", main = "Comparison of hydropathy score results",
    las = 1)
abline(c(0, -1), col = "red")
abline(v = 0, lty = 2)
abline(h = 0, lty = 2)
```



The results are consistent, it's hard to say whether the small differences are due to Excel rounding errors or because the method used to compute the GRAVY score was not exactly the same (in [17] they used the mean over a sliding window).

2.2 Importing aligned sequence data

2.2.1 Aligned sequences files examples

mase

Mase format is a flatfile format use by the SeaView multiple alignment editor [8], developed by Manolo Gouy and available at http://pbil.univ-lyon1.fr/software/seaview.html. The mase format is used to store nucleotide or protein multiple alignments. The beginning of the file must contain a header containing at least one line (but the content of this header may be empty). The header lines must begin by ;;. The body of the file has the following structure: First, each entry must begin by one (or more) commentary line. Commentary lines begin by the character ;. Again, this commentary line may be empty. After the commentaries, the name of the sequence is written on a separate line. At last, the sequence itself is written on the following lines.

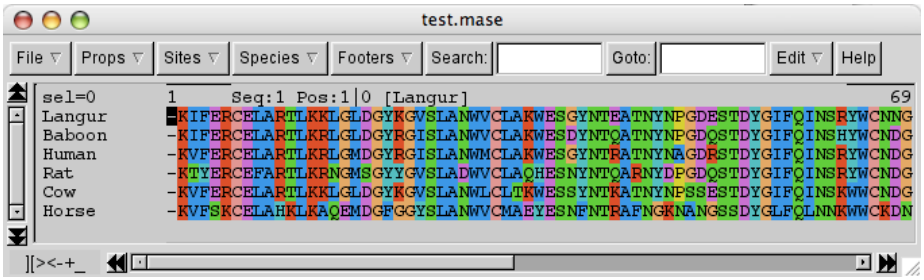


Figure 2.1: The file `test.mase` under SeaView. This is a graphical multiple sequence alignment editor developped by Manolo Gouy [8]. SeaView is able to read and write various alignment formats (NEXUS, MSF, CLUSTAL, FASTA, PHYLIP, MASE). It allows to manually edit the alignment, and also to run DOT-PLOT or CLUSTALW programs to locally improve the alignment.

```
masef <- system.file("sequences/test.mase", package = "seqinr")
cat(readLines(masef), sep = "\n")
```

```
;;Aligned by clustal on Tue Jun 30 17:36:11 1998
;empty description
Langur
-KIFERCELARTLKKLGLDGYKGVSLANWVCLAKWESGYNTEATNYNPGDESTDYGIFQINSRYWCNNGKPGAVDACHISCSALLQNNIADAVACAKRVVSDQGIRAWVAWRNHCQN
;
Baboon
-KIFERCELARTLKRLGLDGYRGISLANWVCLAKWESDYNTQATNYNPGDQSTDYGIFQINSHYWCNDGKPGAVNACHISCNALLQDNITDAVACAKRVVSDQGIRAWVAWRNHCQN
;
Human
-KVFERCELARTLKRLGMDGYRGISLANWMCLAKWESGYNTRATNYNAGDRSTDYGIFQINSRYWCNDGKPGAVNACHLSCSALLQDNIADAVACAKRVVRDQGIRAWVAWRNRCQN
;
Rat
-KTYERCEFARTLKRNGMSGYYGVSLADWVCLAQHESNYNTQARNYDPGDQSTDYGIFQINSRYWCNDGKPRAKNACGIPCSALLQDDITQAIQCAKRVVRDQGIRAWVAWQRHCKN
;
Cow
-KVFERCELARTLKKLGLDGYKGVSLANWLCLTKWESSYNTKATNYNPSSESTDYGIFQINSKWWCNDGKPNAVDGCHVSCSELMENDIAKAVACAKKIVSEQGITAWVAWKSHCRD
;
Horse
-KVFSKCELAHKLKAQEMDGFGGYSLANWVCMAEYESNFNTRAFNGKNANGSSDYGLFQLNNKWWCKDNKRSSSNACNIMCSKLLDENIDDDISCAKRVVRDKGMSAWKAWVKHCKD
```

A screenshot copy of the same file as seen under SeaView is given in figure 2.1.

clustal

The CLUSTAL format (*.aln) is the format of the ClustalW multialignment tool output [18, 57]. It can be described as follows. The word CLUSTAL is on the first line of the file. The alignment is displayed in blocks of a fixed length, each line in the block corresponding to one sequence. Each line of each block starts with the sequence name (maximum of 10 characters), followed by at least one space character. The sequence is then displayed in upper or lower cases, '-' denotes gaps. The residue number may be displayed at the end of the first line of each block.

```
clustalf <- system.file("sequences/test.aln", package = "seqinr")
cat(readLines(clustalf), sep = "\n")
```

```
CLUSTAL W (1.82) multiple sequence alignment


FOSB_MOUSE      MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVPTVTA 60
FOSB_HUMAN      MFQAFPGDYDSGSRCSSSPSAESQYLSSVDSFGSPPTAAASQECAGLGEMPGSFVPTVTA 60
                ************************************************************

FOSB_MOUSE      ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPAVDPYDMPGTSYSTPGLSAYSTGGASGS 120
FOSB_HUMAN      ITTSQDLQWLVQPTLISSMAQSQGQPLASQPPVVDPYDMPGTSYSTPGMSGYSSGGASGS 120
                *******************************.***************:*.**:******

FOSB_MOUSE      GGPSTSTTTSGPVSARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRRRELT 180
FOSB_HUMAN      GGPSTSGTTSGPGPARPARARPRRPREETLTPEEEEKRRVRRERNKLAAAKCRNRRRELT 180
                ****** ***** .********************************************

FOSB_MOUSE      DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPLAEVRD 240
FOSB_HUMAN      DRLQAETDQLEEEKAELESEIAELQKEKERLEFVLVAHKPGCKIPYEEGPGPGPLAEVRD 240
                ************************************************************

FOSB_MOUSE      LPGSTSAKEDGFGWLLPPPPPPPLPFQSSRDAPPNLTASLFTHSEVQVLGDPFPVVSPSY 300
FOSB_HUMAN      LPGSAPAKEDGFSWLLPPPPPPPLPFQTSQDAPPNLTASLFTHSEVQVLGDPFPVVNPSY 300
                ****:.******.**************:*:**************************.***

FOSB_MOUSE      TSSFVLTCPEVSAFAGAQRTSGSEQPSDPLNSPSLLAL 338
FOSB_HUMAN      TSSFVLTCPEVSAFAGAQRTSGSDQPSDPLNSPSLLAL 338
                **********************:**************
```

phylip

PHYLIP is a tree construction program [6]. The format is as follows: the number of sequences and their length (in characters) is on the first line of the file. The alignment is displayed in an interleaved or sequential format. The sequence names are limited to 10 characters and may contain blanks.

```
phylipf <- system.file("sequences/test.phylip", package = "seqinr")
cat(readLines(phylipf), sep = "\n")
```

```
  5    42
Turkey     AAGCTNGGGC ATTTCAGGGT
Salmo gairAAGCCTTGGC AGTGCAGGGT
H. SapiensACCGGTTGGC CGTTCAGGGT
Chimp      AAACCCTTGC CGTTACGCTT
Gorilla    AAACCCTTGC CGGTACGCTT

GAGCCCGGGC AATACAGGGT AT
GAGCCGTGGC CGGGCACGGT AT
ACAGGTTGGC CGTTCAGGGT AA
AAACCGAGGC CGGGACACTC AT
AAACCATTGC CGGTACGCTT AA
```

msf

MSF is the multiple sequence alignment format of the GCG sequence analysis package (http://www.accelrys.com/products/gcg/index.html). It begins with the line (all uppercase) !!NA_MULTIPLE_ALIGNMENT 1.0 for nucleic acid sequences or !!AA_MULTIPLE_ALIGNMENT 1.0 for amino acid sequences. Do not edit or delete the file type if its present (optional). A description line which contains informative text describing what is in the file. You can add this information to the top of the MSF file using a text editor (optional). A dividing line which contains the number of bases or residues in the sequence, when the file was created, and importantly, two dots (..) which act as a divider between the descriptive information and the following sequence information (required). msf files contain some other information: the Name/Weight, a Separating Line which must include two slashes (//) to divide the name/weight

information from the sequence alignment (required) and the multiple sequence alignment.

```
msff <- system.file("sequences/test.msf", package = "seqinr")
cat(readLines(msff), sep = "\n")
```

```
PileUp of: @Pi3k.Fil

Symbol comparison table: GenRunData:Pileuppep.Cmp  CompCheck: 1254

                  GapWeight: 3.000
              GapLengthWeight: 0.100

 Pi3k.Msf  MSF: 377  Type: P  July 12, 1996 10:40  Check: 167 ..

 Name: Tor1_Yeast       Len:   377  Check: 7773  Weight:  1.00
 Name: Tor2_Yeast       Len:   377  Check: 8562  Weight:  1.00
 Name: Frap_Human       Len:   377  Check: 9129  Weight:  1.00
 Name: Esr1_Yeast       Len:   377  Check: 8114  Weight:  1.00
 Name: Tel1_Yeast       Len:   377  Check: 1564  Weight:  1.00
 Name: Pi4k_Human       Len:   377  Check: 8252  Weight:  1.00
 Name: Stt4_Yeast       Len:   377  Check: 9117  Weight:  1.00
 Name: Pik1_Yeast       Len:   377  Check: 3455  Weight:  1.00
 Name: P3k1_Soybn       Len:   377  Check: 4973  Weight:  1.00
 Name: P3k2_Soybn       Len:   377  Check: 4632  Weight:  1.00
 Name: Pi3k_Arath       Len:   377  Check: 3585  Weight:  1.00
 Name: Vp34_Yeast       Len:   377  Check: 5928  Weight:  1.00
 Name: P11a_Human       Len:   377  Check: 6597  Weight:  1.00
 Name: P11b_Human       Len:   377  Check: 8486  Weight:  1.00

//

            1                                                  50
Tor1_Yeast  .......GHE DIRQDSLVMQ LFGLVNTLLK NDSECFKRHL DIQQYPAIPL
Tor2_Yeast  .......GHE DIRQDSLVMQ LFGLVNTLLQ NDAECFRRHL DIQQYPAIPL
Frap_Human  .......GHE DLRQDERVMQ LFGLVNTLLA NDPTSLRKNL SIQRYAVIPL
Esr1_Yeast  .......KKE DVRQDNQYMQ FATTMDFLLS KDIASRKRSL GINIYSVLSL
Tel1_Yeast  .KALMKGSND DLRQDAIMEQ VFQQVNKVLQ NDKVLRNLDL GIRTYKVVPL
Pi4k_Human  ..AAIFKVGD DCRQDMLALQ IIDLFKNIFQ LV....GLDL FVFPYRVVAT
Stt4_Yeast  ..AAIFKVGD DCRQDVLALQ LISLFRTIWS SI....GLDV YVFPYRVTAT
Pik1_Yeast  ...VIAKTGD DLRQEAFAYQ MIQAMANIWV KE....KVDV WVKRMKILIT
P3k1_Soybn  TCKIIFKKGD DLRQDQLVVQ MVSLMDRLLK LE....NLDL HLTPYKVLAT
P3k2_Soybn  ....IFKKGD DIRQDQLVVQ MVSLMDRLLK LE....NLDL HLTPYKVLAT
Pi3k_Arath  ..KLIFKKGD DLRQDQLVVQ MVWLMDRLLK LE....NLDL CLTPYKVLAT
Vp34_Yeast  .YHLMFKVGD DLRQDQLVVQ IISLMNELLK NE....NVDL KLTPYKILAT
P11a_Human  ...IIFKNGD DLRQDMLTLQ IIRIMENIWQ NQ....GLDL RMLPYGCLSI
P11b_Human  ...VIFKNGD DLRQDMLTLQ MLRLMDLLWK EA....GLDL RMLPYGCLAT

            51                                                 100
Tor1_Yeast  SPKSGLLGWV PNSDTFHVLI REHRDAKKIP LNIEHWVMLQ MAPDYENLTL
Tor2_Yeast  SPKSGLLGWV PNSDTFHVLI REHREAKKIP LNIEHWVMLQ MAPDYDNLTL
Frap_Human  STNSGLIGWV PHCDTLHALI RDYREKKKIL LNIEHRIMLR MAPDYDHLTL
Esr1_Yeast  REDCGILEMV PNVVTLRSIL STKYESLKIK Y.....SLKS LHDRWQHTAV
Tel1_Yeast  GPKAGIIEFV ANSTSLHQIL SKLHTNDKIT FDQARKGMKA VQTKSN....
Pi4k_Human  APGCGVIECI PDCTS..... .......... RDQLGRQTDF GMYDYFTRQY
Stt4_Yeast  APGCGVIDVL PNSVS..... .......... RDMLGREAVN GLYEYFTSKF
Pik1_Yeast  SANTGLVETI TNAMSVHSIK KALTKKMIED AELDDKGGIA SLNDHFLRAF
P3k1_Soybn  GQDEGMLEFI P.SRSLAQI. .......... ..LSENRSII SYLQ......
P3k2_Soybn  GQDEGMLEFI P.SRSLAQI. .......... ..LSENRSII SYLQ......
Pi3k_Arath  GHDEGMLEFI P.SRSLAQI. .......... ..LSEHRSIT SYLQ......
Vp34_Yeast  GPQEGAIEFI P.NDTLASI. .......... ..LSKYHGIL GYLK......
P11a_Human  GDCVGLIEVV RNSHTIMQI. .......... ..Q.CKGGLK GALQFNSHTL
P11b_Human  GDRSGLIEVV STSETIADI. .......... ..QLNSSNVA AAAAFNKDAL
```

FASTA

Sequence in fasta format begins with a single-line description (distinguished by a greater-than (>) symbol), followed by sequence data on the next line.

```
fastaf <- system.file("sequences/Anouk.fasta", package = "seqinr")
cat(readLines(fastaf), sep = "\n")
```

```
>LmjF01.0030
ATGATGTCGGCCGAGCCGCCGTCGTCGCAGCCGTACATCAGCGACGTGCTGCGGCGGTAC
CAGCTGGAGCGCTTTCAGTGTGCCTTTGCATCGAGCATGACCATCAAGGACCTCCTCGCC
CTGCAGCCAGAGGACTTCAACCGCTACGGCGTCGTAGAGGCGATGGACATTTTGCGGCTG
CGTGACGCCATCGAGTACATCAAGGCTAATCCGCTCCCCGCCTCGCGCTCTGGCAGTGAC
GTGCTCGACAACGACGGCGACGGCGACGGCGACGACAGTACGCCGGAGGGGAAGGAGGGG
TGCTCGACGGAGCGCCGGCGGCAGTACACAGCACGCGGAACCACAGTCCTTTGCCGGTCG
ACCGACACCGCCGAGGAGGTGAAGCGCAAGAGCCGCATCCTCGTCGCCATTCGCAAGCGT
CCGCTCAGCGCCGGGGAGCAGACGAACGGCTTCACGGACATCATGGACGCCGACAACAGC
GGCGAGATTGTGCTGAAGGAGCCAAAGGTGAAGGTCGACCTCCGCAAGTACACCCACGTG
CACCGCTTCTTCTTCGACGAGGTTTTCGACGAGGCCTGCGACAACGTCGACGTGTACAAC
CGCGCTGCCCGCGCGCTGATCGACACCGTCTTCGACGGCGGCTGCGCGACATGCTTCGCC
TATGGACAGACAGGGAGCGGCAAGACACACACGATGCTGGGCAAGGGCCCCGAGCCGGGC
CTCTACGCACTCGCCGCCAAAGACATGTTTGACCGCCTCACGAGCGACACGCGCATCGTC
GTTTCCTTTTACGAGATCTACAGCGGGAAGCTCTTTGACTTGCTGAACGGCCGGCGACCC
CTGCGAGCCCTCGAGGACGACAAGGGCCGGGTGAACATCCGCGGCCTCACCGAAACACTGC
TCTACCAGCGTGGAGGACCTCATGACGATCATCGACCAGGGCAGCGGTGTTCGCAGCTGC
GGCTCCACCGGCGCCAATGACACAAGCTCCCGCTCCCACGCCATTCTCGAGATCAAGCTC
AAGGCGAAACGGACGTCGAAGCAGAGCGGCAAGTTCACGTTCATCGACCTCGCTGGAAGC
GAGCGCGGCGCTGACACGGTGGACTGCGCGCGACAGACACGCCTCGAAGGGGCGGAGATC
AACAAGAGCCTACTCGCGCTGAAGGAGTGCATTCGTTTTTTAGATCAGAACAGGAAGCAC
GTCCCGTTCCGCGGCTCGAAGCTGACTGAGGTGCTCCGCGACTCGTTTATCGGCAACTGC
CGCACGGTGATGATCGGCGCCGTCTCTCCGTCGAACAACAATGCCGAGCACACGCTGAAC
ACGCTGCGCTACGCCGATCGTGTCAAGGAGCTGAAGCGCAACGCCACGGAGCGGCGCACT
GTGTGCATGCCCGACGACCAGGAAGAGGCCTTCTTTGACACGACCGAGAGCAGGCCACCG
TCGCGGAGGACGACAACTCGCCTTTCTACGGCCGCCCCGCTTTTCTCCGGCTCTTCGACG
GCTGCGCCAGCACTTAGAAGCACGCTACTCAGCAGCCGCTCCGTCAACACACTCTCGCCG
TCGTCGCAGGCCAAGTCGACTCTCGTCACCCCGAAGCCGCCGTCGCGCGATCGGACTCCG
GACATGGTGTGCACTAAGCGGCCCCGCGACTCAGACAGAAGCGGCGAGGACGAAGTGGTA
GCGCGGCCGAGTGGGCGCCCAAGCTTCAAGCGCTTCGAGAGCGGCGCCGAGCTTGTCGCG
GCCCAGCGCAGTCGCGTCATTGACCAATACAACGCCTACCTCGAGACGGACATGAACTGT
ATCAAGGAGGAGTACCAGGTGAAGTACGACGCAGAGCAGATGAACGCCAACACGCGCAGC
TTTGTGGAGCGCGCACGTCTGCTGGTGAGCGAGAAACGGCGCGCGATGGAGTCCTTCCTA
ACGCAGCTGGAGGAGCTCGACAAGATCGCGCAGCAGGTCGCCGACATCACCGCCTTTCAG
CAGCACCTGCCGCCAACG
>LinJ01.0030
ATGATGTCGGCCGAGCCGCCGTCGTCGCAGCCGTACATCAGCGACGTGCTGCGGCGGTAC
CAGCTGGAGCGCTTTCAGAGTTCCTTTGCATCGAGCATGACCATCAAGGACCTCCTCGCC
CTGCAGCCGGAGGACTTCAACCGCTACGGCGTCGTAGAGGCAATGGACATTTTGCGGCTG
CGCGACGCCATCGAGTACATCAAGGCCAACCCGCTCCCCGCCTCGCGCTCCGGCAGTGAC
GTGCTCGACAACGACGGCGACGGCGACGGCGACGACAGTACGCCGGAGGGGAAGGAGGGG
TGCTCGACGGAGCGCCGACGGCAGTACACAGCACGCGGAACCACCGTCCTTTGCGGGTCG
ACCGACACCGCCGAGGAGGTGAAGCGCAAGAGCCGCATCATCGTCGCCATTCGCAAGCGT
CCGCTCAGCGCCGGGGAGCAGACGAACGGCTTCACGGACATCATGGACGCCGACAACAAC
GGCGAGATTGTGCTGAAGGAGCCAAAGGTGAAGGTCGACCTCCGCAAGTACACCCACGTG
CACCGCTTCTTCTTCGACGAGGTTTTCGACGAGGCGTGCGACAACGTCGACGTGTACAAC
CGCGCTGCCCGCGCGCTGATCGACACCGTCTTCGACGGCGGCTGCGCGACATGCTTCGCC
TATGGGCAGACAGGGAGCGGCAAGACACACACGATGCTCGGCAAGGGCCCCGAGCCGGGC
CTGTACGCACTCGCCGCCAAAGACATGTTTGACCGCCTCACGAGCGACACGCGCATCGTT
GTTTCCTTTTACGAGATCTACAGCGGGAAGCTCTTTGACTTGCTGAACGGCCGGCGACCA
CTGCGAGCCCTCGAGGACGACAAGGGGAGGGTGAACATCCGCGGCCTCACCGAACACTGC
TCTACCAGCGTGGAGGACCTCATGACGATCATCGACCAGGGCAGCGGCGTTCGCAGCTGC
GGCTCCACCGGCGCCAACGACACGAGCTCCCGCTCCCACGCCATTCTCGAGATCAAGCTC
AAGGCGAAACGGACGTCGAAGCAGAGCGGCAAGTTCACATTCATCGACCTCGCTGGAAGC
GAGCGCGGCGCCGACACGGTGGATTGCGCGCGACAGACACGCCTCGAAGGGGCGGAGATT
AACAAGAGCCTACTCGCTCTGAAGGAGTGCATTCGTTTTTTAGATCAGAACAGGAAGCAC
GTCCCGTTCCGCGGCTCGAAGCTGACTGAGGTGCTCCGCGACTCGTTTATCGGCAACTGC
CGCACGGTGATGATCGGCGCCGTCTCTCCGTCCAACAACAATGCCGAGCACACGCTGAAC
ACGTTGCGCTACGCCGATCGCGTCAAGGAGCTGAAGCGCAACGCCACGGAGCGGCGCACC
GTGTGCGTGCCCAACGACCAGGAAGAGGCCTTCTTTGACACGACCGAGAGCAGGCCACCG
TCGCGGAGGACGACAACTCGGCTTTCTGCGGCCGCCCCGCTTTTCTCCGGCACTTCGACG
GCTGCCCCAGCATGTAAAAGCACGTTGCTCAGCAGCCGCTCCGTCAACACACTCTCGCCG
TCGTCGCAGGGCAAGTCGACTCTCGTCACCCCGAAGCCACTGTCGCGCGATCGGACTCCG
GACATGGTGTGCGCTAAGCGGCCCCGCGACTCAGACCGAAGCGGCGAAGACGAAGTGGTG
GCGCGGCCGAGTGGGCGCCCAAGCTTCAAGCGCTTCGAGGGCGGCGCCGAGCTCGTGGCG
GCCCAGCGCAGTCGTGTCATTGACCAATACAACGCCTACCTCGAGACGGACATGAACTGT
ATCAAGGAGGAGTACCAGGTGAAGTACGACGCAGAGCAGATGAACGCCAACACGCGCACC
TTTGTCGAGCGCGCACGCCTGCTGGTGAGCGAGAAGCGGCGCGCGATGGAGTCCTTCCTA
ACGCAGCTGGACGAGCTCGATAAGATCGCGCAGCAGGTCGCCAGCATCACCGCCTTTCAG
CAGCACCTGCCGCCAACG
```

2.2.2 The function `read.alignment()`

Aligned sequence data are very important in evolutionary studies, in this representation all vertically aligned positions are supposed to be homologous, that is sharing a common ancestor. This is a mandatory starting point for comparative studies. There is a function in **seqinR** called `read.alignment()` to read

aligned sequences data from various formats (`mase`, `clustal`, `phylip`, `fasta` or `msf`) produced by common external programs for multiple sequence alignment.

```
example(read.alignment)
```

```
rd.lgn> mase <- read.alignment(File = system.file("sequences/test.mase",
    package = "seqinr"), format = "mase")
rd.lgn> clustal <- read.alignment(File = system.file("sequences/test.aln",
    package = "seqinr"), format = "clustal")
rd.lgn> phylip <- read.alignment(File = system.file("sequences/test.phylip",
    package = "seqinr"), format = "phylip")
rd.lgn> msf <- read.alignment(File = system.file("sequences/test.msf",
    package = "seqinr"), format = "msf")
rd.lgn> fasta <- read.alignment(File = system.file("sequences/Anouk.fasta",
    package = "seqinr"), format = "fasta")
```

2.2.3 A simple example with the louse-gopher data

Let's give an example. The gene coding for the mitochondrial cytochrome oxidase I is essential and therefore often used in phylogenetic studies because of its ubiquitous nature. The following two sample tests of aligned sequences of this gene (extracted from ParaFit [30]), are distributed along with the **seqinR** package:

```
louse <- read.alignment(system.file("sequences/louse.fasta",
    package = "seqinr"), format = "fasta")
louse$nam
```

```
[1] "gi|548117|gb|L32667.1|GYDCYTOXIB" "gi|548119|gb|L32668.1|GYDCYTOXIC"
[3] "gi|548121|gb|L32669.1|GYDCYTOXID" "gi|548125|gb|L32671.1|GYDCYTOXIF"
[5] "gi|548127|gb|L32672.1|GYDCYTOXIG" "gi|548131|gb|L32675.1|GYDCYTOXII"
[7] "gi|548133|gb|L32676.1|GYDCYTOXIJ" "gi|548137|gb|L32678.1|GYDCYTOXIL"
```

```
gopher <- read.alignment(system.file("sequences/gopher.fasta",
    package = "seqinr"), format = "fasta")
gopher$nam
```

```
[1] "gi|548223|gb|L32683.1|PPGCYTOXIA" "gi|548197|gb|L32686.1|OGOCYTOXIA"
[3] "gi|548199|gb|L32687.1|OGOCYTOXIB" "gi|548201|gb|L32691.1|OGOCYTOXIC"
[5] "gi|548203|gb|L32692.1|OGOCYTOXID" "gi|548229|gb|L32693.1|PPGCYTOXID"
[7] "gi|548231|gb|L32694.1|PPGCYTOXIE" "gi|548205|gb|L32696.1|OGOCYTOXIE"
```

The aligned sequences are now imported in your R environment. The 8 genes of the first sample are from various species of louse (insects parasitics on warm-blooded animals) and the 8 genes of the second sample are from their corresponding gopher hosts (a subset of rodents), see figure 2.2 :

```
l.names <- readLines(system.file("sequences/louse.names",
    package = "seqinr"))
l.names
```

```
[1] "G.chapini "  "G.cherriei " "G.costaric " "G.ewingi  "  "G.geomydis "
[6] "G.oklahome " "G.panamens " "G.setzeri  "
```

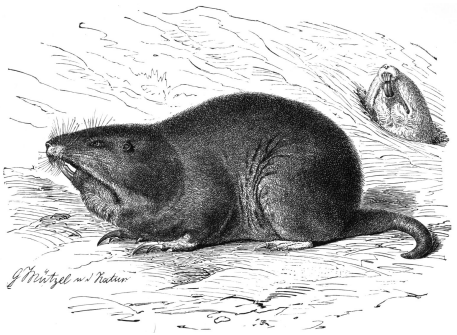


Figure 2.2: Louse (left) and gopher (right). Images are from the wikipedia (http://www.wikipedia.org/). The picture of the chewing louse *Damalinia limbata* found on Angora goats was taken by Fiorella Carnevali (ENEA, Italy). The gopher drawing is from Gustav Mützel, Brehms Tierleben, Small Edition 1927.

```
g.names <- readLines(system.file("sequences/gopher.names",
    package = "seqinr"))
g.names
```

```
[1] "G.brevicep " "O.cavator "  "O.cherriei " "O.underwoo " "O.hispidus "
[6] "G.burs1 "    "G.burs2 "    "O.heterodu"
```

SeqinR has very few methods devoted to phylogenetic analyses but many are available in the ape package. This allows for a very fine tuning of the graphical outputs of the analyses thanks to the power of the R facilities. For instance, a natural question here would be to compare the topology of the tree of the hosts and their parasites to see if we have congruence between host and parasite evolution. In other words, we want to display two phylogenetic trees face to face. This would be tedious with a program devoted to the display of a single phylogenetic tree at time, involving a lot of manual copy/paste operations, hard to reproduce, and then boring to maintain with data updates.

How does it looks under R? First, we need to *infer* the tree topologies from data. Let's try as an *illustration* the famous neighbor-joining tree estimation of Saitou and Nei [49] with Jukes and Cantor's correction [24] for multiple substitutions.

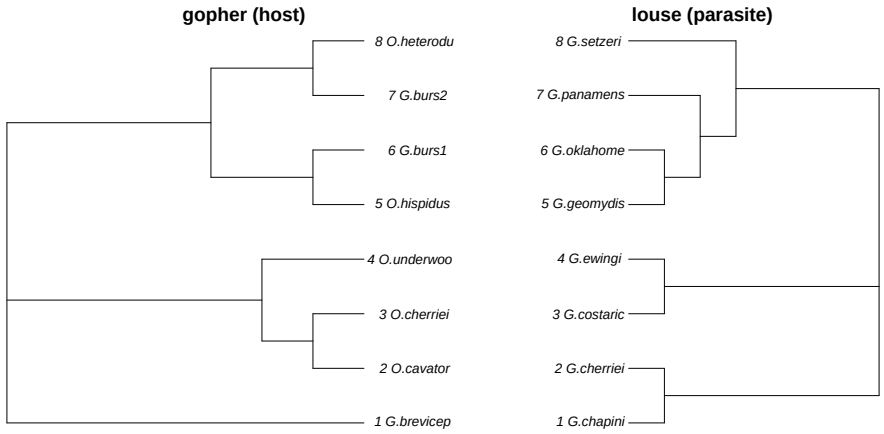
```
library(ape)
louse.JC <- dist.dna(x = lapply(louse$seq, s2c), model = "JC69")
gopher.JC <- dist.dna(x = lapply(gopher$seq, s2c), model = "JC69")
l <- nj(louse.JC)
g <- nj(gopher.JC)
```

Now we have an estimation for *illustrative* purposes of the tree topology for the parasite and their hosts. We want to plot the two trees face to face, and for this we must change R graphical parameters. The first thing to do is to save the current graphical parameter settings so as to be able to restore them later:

```
op <- par(no.readonly = TRUE)
```

The meaning of the `no.readonly = TRUE` option here is that graphical parameters are not all settable, we just want to save those we can change at will. Now, we can play with graphics :

```
g$tip.label <- paste(1:8, g.names)
l$tip.label <- paste(1:8, l.names)
layout(matrix(data = 1:2, nrow = 1, ncol = 2), width = c(1.4,
    1))
par(mar = c(2, 1, 2, 1))
plot(g, adj = 0.8, cex = 1.4, use.edge.length = FALSE, main = "gopher (host)",
    cex.main = 2)
plot(l, direction = "l", use.edge.length = FALSE, cex = 1.4,
    main = "louse (parasite)", cex.main = 2)
```



We now restore the old graphical settings that were previously saved:

```
par(op)
```

OK, this may look a little bit obscure if you are not fluent in programming, but please try the following experiment. In your current working directory, that is in the directory given by the `getwd()` command, create a text file called `essai.r` with your favourite text editor, and copy/paste the previous R commands, that is :

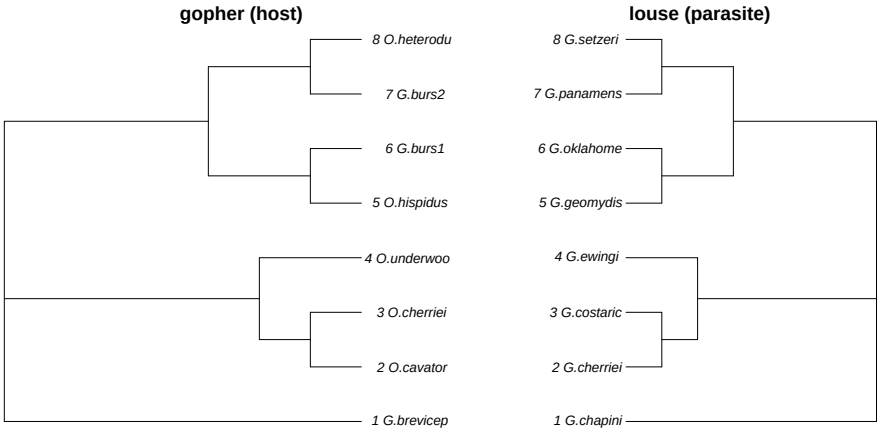
```
louse <- read.alignment(system.file("sequences/louse.fasta", package = "seqinr"), format = "fasta")
gopher <- read.alignment(system.file("sequences/gopher.fasta", package = "seqinr"), format = "fasta")
l.names <- readLines("http://pbil.univ-lyon1.fr/software/SeqinR/Datasets/louse.names")
g.names <- readLines("http://pbil.univ-lyon1.fr/software/SeqinR/Datasets/gopher.names")
louse.JC <- dist.dna(x =  lapply(louse$seq, s2c), model = "JC69" )
gopher.JC <- dist.dna(x =  lapply(gopher$seq, s2c), model = "JC69" )
l <- nj(louse.JC)
g <- nj(gopher.JC)
g$tip.label <- paste(1:8, g.names)
l$tip.label <- paste(1:8, l.names)
layout(matrix(data = 1:2, nrow = 1, ncol = 2), width=c(1.4, 1))
par(mar=c(2,1,2,1))
plot(g, adj = 0.8, cex = 1.4, use.edge.length=FALSE,
  main = "gopher (host)", cex.main = 2)
plot(l,direction="l", use.edge.length=FALSE, cex = 1.4,
  main = "louse (parasite)", cex.main = 2)
```

Make sure that your text has been saved and then go back to R console to enter the command :

```
source("essai.r")
```

This should reproduce the previous face-to-face phylogenetic trees in your R graphical device. Now, your boss is unhappy with working with the Jukes and

Cantor's model [24] and wants you to use the Kimura's 2-parameters distance [28] instead. Go back to the text editor to change `model = "JC69"` by `model = "K80"`, save the file, and in the R console `source("essai.r")` again, you should obtain the following graph :



Nice congruence, isn't it? Now, something even worst, there was a error in the aligned sequence set : the first base in the first sequence in the file `louse.fasta` is not a C but a T. To locate the file on your system, enter the following command:

```
system.file("sequences/louse.fasta", package = "seqinr")
```

```
[1] "../../../../../seqinr.Rcheck/seqinr/sequences/louse.fasta"
```

Open the `louse.fasta` file in your text editor, fix the error, go back to the R console to `source("essai.r")` again. That's all, your graph is now consistent with the updated dataset.

2.3 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

CHAPTER 3

Importing sequences from ACNUC databases

Charif, D. Lobry, J.R.

Contents

Introduction

As a rule of thumb, after compression one nucleotide needs one octet of disk space storage (because you need also the annotations corresponding to the sequences), so that most likely you won't have enough space on your computer to work with a local copy of a complete DNA database. The idea is to import under R only the subset of sequences you are interested in. This is done in three steps detailed thereafter.

3.1 Choose a bank

Select the database from which you want to extract sequences with the `choosebank()` function. This function initiates a remote access to an ACNUC database. Called without arguments, `choosebank()` returns the list of available databases:

```
choosebank()
```

```
[1] "genbank"      "embl"         "emblwgs"      "swissprot"
[5] "ensemblold"   "ensembl"      "emglib"       "nrsub"
```

```
 [9] "nbrf"          "hobacnucl"    "hobacprot"    "hovernucl"
[13] "hoverprot"     "hogennucl"    "hogenprot"    "hoverclnu"
[17] "hoverclpr"     "homolensprot" "homolensnucl" "HAMAPnucl"
[21] "HAMAPprot"     "hoppsigen"    "nurebnucl"    "nurebprot"
[25] "taxobacgen"    "greview"      "hogendnucl"   "hogendprot"
[29] "refseq"
```

Biological sequence databases are fast moving targets, and for publication purposes it is recommended to specify on which release you were working on when you made the job. To get more informations about available databases on the server, just set the `infobank` parameter to `TRUE`. For instance, here is the result for the three first databases on the default server at the compilation time (September 1, 2006) of this document:

```
choosebank(infobank = TRUE)[1:3, ]
```

```
     bank status
1 genbank     on
2    embl     on
3 emblwgs     on
                                                          info
1 GenBank Rel. 155 (15 August 2006) Last Updated: Sep  1, 2006
2                          EMBL Library Release 87 (June 2006)
3  EMBL Whole Genome Shotgun sequences Release 87  (June 2006)
```

Note that there is a `status` column because a database could be unavailable for a while during updates. If you try call `choosebank(bank = "bankname")` when the bank called `bankname` is off from server, you will get an explicit error message stating that this bank is temporarily unavailable, for instance:

```
choosebank("off")
```

```
Error in choosebank("off") : Database with name -->off<-- is currently off for maintenance,
please try again later.
```

Some special purpose databases are not listed by default. These are *tagged* databases that are only listed if you provide an explicit `tagbank` argument to the `choosebank()` function. Of special interest for teaching purposes is the `TP` tag, an acronym for *Travaux Pratiques* which means "practicals", and corresponds to *frozen* databases so that you can set up a practical whose results are stable from year to year. Currently available frozen databases at the default server are:

```
choosebank(tagbank = "TP", infobank = TRUE)
```

```
         bank status
1      emblTP     on
2 swissprotTP     on
3 hoverprotTP     on
4 hovernuclTP     on
5     trypano     on
                                                                    info
1                          EMBL Library Release 78 WITHOUT ESTs  (March 2004)
2 UniProt Rel. 1 (SWISS-PROT 43 + TrEMBL 26 + NEW): Last Updated: May  3, 2004
3               HOVERGEN - Release 45 (Jan 22 2004) Last Updated: Jan 22, 2004
4               HOVERGEN - Release 45 (Jan 22 2004) Last Updated: Jan 22, 2004
5                  trypano Rel. 1 (27 Janvier 2004) Last Updated: Jan 27, 2004
```

Now, if you want to work with a given database, say GenBank, just call `choosebank()` with `"genbank"` as its first argument and store the result in a variable in the workspace, called for instance `mybank` in the example thereafter:

```
mybank <- choosebank("genbank")
str(mybank)
```

```
List of 8
 $ socket  :Classes 'sockconn', 'connection'  int 8
 $ bankname: chr "genbank"
 $ totseqs : chr "64946744"
 $ totspecs: chr "393311"
 $ totkeys : chr "2109217"
 $ release : chr "GenBank Rel. 155 (15 August 2006) Last Updated: Sep  1, 2006"
 $ status  :Class 'AsIs'  chr "on"
 $ details : chr [1:3] "GenBank Rel. 155 (15 August 2006) Last Updated: Sep  1, 2006" "66,009,892,298 bases; 61,704,1
```

The list returned by `choosebank()` here means that in the database called `genbank` at the compilation time of this document there were `64,946,744` sequences from `393,311` species and a total of `2,109,217` keywords. The status of the bank was `on`, and the release information was `GenBank Rel. 155 (15 August 2006) Last Updated: Sep 1, 2006`. For specialized databases, some relevant informations are also given in the `details` component, for instance:

```
choosebank("taxobacgen")$details
```

```
 [1] "TaxoBacGen Rel. 7 (September 2005)"
 [2] "1,151,149,763 bases; 254,335 sequences; 847,767 subseqs; 63,879 refers."
 [3] "Data compiled from GenBank by Gregory Devulder"
 [4] "Laboratoire de Biometrie & Biologie Evolutive, Univ Lyon I"
 [5] "------------------------------"
 [6] "This database is a taxonomic genomic database."
 [7] "It results from an expertise crossing the data nomenclature database DSMZ"
 [8] "[http://www.dsmz.de/species/bacteria.htm Deutsche Sammlung von"
 [9] "Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany]"
[10] "and GenBank."
[11] "- Only contains sequences described under species present in"
[12] "Bacterial Nomenclature Up-to-date."
[13] "- Names of species and genus validly published according to the"
[14] "Bacteriological Code (names with standing in nomenclature) is"
[15] "added in field \"DEFINITION\"."
[16] "- A keyword \"type strain\" is added in field \"FEATURES/source/strain\" in"
[17] "GenBank format definition to easyly identify Type Strain."
[18] "Taxobacgen is a genomic database designed for studies based on a strict"
[19] "respect of up-to-date nomenclature and taxonomy."
```

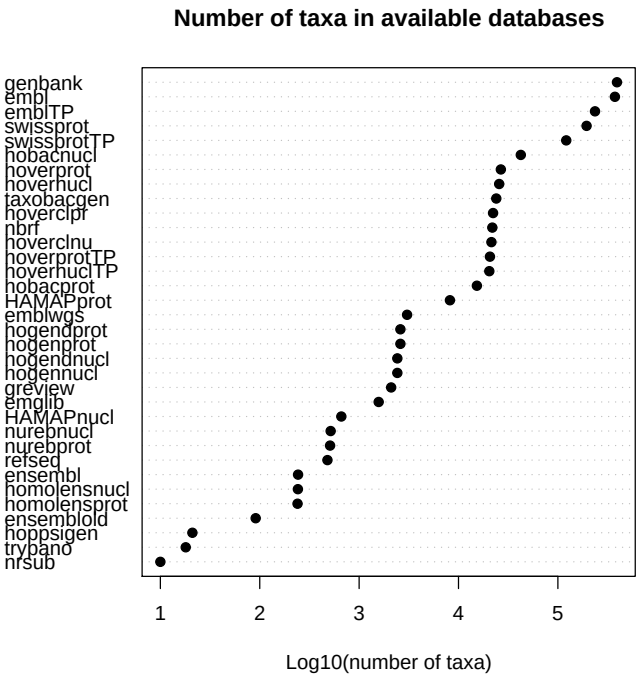
The previous command has a side-effect that is worth mentioning. As from **seqinR** 1.0-3, the result of the `choosebank()` function is automatically stored in a global variable named `banknameSocket`, so that if no socket argument is given to the `query()` function, the last opened database will be used by default for your requests. This is just a matter of convenience so that you don't have to explicitly specify the details of the socket connection when working with the last opened database. You have, however, full control of the process since `choosebank()` returns (invisibly) all the required details. There is no trouble to open *simultaneously* many databases. You are just limited by the number of simultaneous connections your build of R is allowed[1].

For advanced users who may wish to access to more than one database at time, a good advice is to close them with the function `closebank()` as soon as possible so that the maximum number of simultaneous connections is never reached. In the example below, we want to display the number of taxa (*i.e.*

[1] There is a very convenient function called `closeAllConnections()` in the R base package if you want to close all open connections at once.

the number of nodes) in the species taxonomy associated with each available database (including frozen databases). For this, we loop over available databases and close them as soon as the information has been retrieved.

```
banks <- c(choosebank(), choosebank(tagbank = "TP"))
ntaxa <- numeric(0)
for (i in banks) {
    ntaxa[i] <- as.numeric(choosebank(i)$totspecs)
    closebank()
}
dotchart(log10(ntaxa[order(ntaxa)]), pch = 19, main = "Number of taxa in available databases",
    xlab = "Log10(number of taxa)")
```



3.2 Make your query

For this section, set up the default bank to GenBank, so that you don't have to provide the sockets details for the `query()` function:

```
choosebank("genbank")
```

Then, you have to say what you want, that is to compose a query to select the subset of sequences you are interested in. The way to do this is documented under `?query`, we just give here a simple example. In the query below, we want to select all the coding sequences (`t=cds`) from cat (`sp=felis catus`) that are not (`et no`) partial sequences (`k=partial`). We want the result to be stored in an object called `completeCatsCDS`.

```
query("completeCatsCDS", "sp=felis catus et t=cds et no k=partial")
```

Now, there is in the workspace an object called `completeCatsCDS`, which does not contain the sequences themselves but the *sequence names* (and various relevant informations such as the genetic code and the frame) that fit the query. They are stored in the `req` component of the object, let's see the name of the first ten of them:

```
sapply(completeCatsCDS$req[1:10], getName)
```

```
[1] "AB000483.PE1"    "AB000484.PE1"    "AB000485.PE1"    "AB004237"
[5] "AB004238"        "AB009279.PE1"    "AB009280.PE1"    "AB010872.UGT1A1"
[9] "AB011965.SDF-1A" "AB011966.SDF-1B"
```

The first sequence that fit our request is `AB000483.PE1`, the second one is `AB000484.PE1`, and so on. Note that the sequence name may have an extension, this corresponds to *subsequences*, a specificity of the ACNUC system that allows to handle easily a subsequence with a biological meaning, typically a gene. The list of available subsequences in a given database is given by the function `getType()`, for example the list of available subsequences in GenBank is given in table 3.1.

	Type	Description
1	CDS	.PE protein coding region
2	LOCUS	sequenced DNA fragment
3	MISC_RNA	.RN other structural RNA coding region
4	RRNA	.RR mature ribosomal RNA
5	SCRNA	.SC small cytoplasmic RNA
6	SNRNA	.SN small nuclear RNA
7	TRNA	.TR mature transfer RNA

Table 3.1: Available subsequences in genbank

The component `call` of `completeCatsCDS` keeps automatically a trace of the way you have selected the sequences:

```
completeCatsCDS$call
```

```
query(listname = "completeCatsCDS", query = "sp=felis catus et t=cds et no k=partial")
```

At this stage you can quit your R session saving the workspace image. The next time an R session is opened with the workspace image restored, there will be an object called `completeCatsCDS`, and looking into its `call` component will tell you that it contains the names of complete coding sequences from *Felis catus*.

In practice, queries for sequences are rarely done in one step and are more likely to be the result of an iterative, progressively refining, process. An important point is that a list of sequences can be re-used. For instance, we can re-use `completeCatsCDS` to get only the list of sequences that were published in 2004:

```
query("ccc2004", "completeCatsCDS et y=2004")
length(ccc2004$req)
```

```
[1] 57
```

Hence, there were 57 complete coding sequences published in 2004 for *Felis catus* in GenBank.

As from release 1.0-3 of the **seqinR** package, there is new parameter `virtual` which allows to disable the automatic retrieval of information for all list elements. This is interesting for list with many elements, for instance :

```
query("allcds", "t=cds", virtual = TRUE)
allcds$nelem
```

```
[1] 3494449
```

There are therefore `3,494,449` coding sequences in this version of GenBank[2]. It would be long to get all the informations for the elements of this list, so we have set the parameter `virtual` to `TRUE` and the `req` component of the list has not been documented:

```
allcds$req
```

```
[[1]]
[1] NA
```

However, the list can still be re-used[3], for instance we may extract from this list all the sequences from, say, *Mycoplasma genitalium*:

```
query("small", "allcds et sp=mycoplasma genitalium", virtual = TRUE)
small$nelem
```

```
[1] 916
```

There are then `916` elements in the list `small`, so that we can safely repeat the previous query without asking for a virtual list:

```
query("small", "allcds et sp=mycoplasma genitalium")
sapply(small$req, getName)[1:10]
```

```
[1] "AY191424" "AY386807" "AY386808" "AY386809" "AY386810" "AY386811"
[7] "AY386812" "AY386813" "AY386814" "AY386815"
```

Here are some illustrations of using virtual list to answer simple questions about the current GenBank release.

Man. How many sequences are available for our species?

```
query("man", "sp=homo sapiens", virtual = T)
man$nelem
```

```
[1] 10649443
```

There are `10,649,443` sequences from *Homo sapiens*.

Sex. How many sequences are annotated with a keyword starting by sex?

[2] which is stored in the `release` component of the object `banknameSocket` and current value is today (September 1, 2006): `banknameSocket$release = GenBank Rel. 155 (15 August 2006) Last Updated: Sep 1, 2006`.

[3] of course, as long as the socket connection with the server has not been lost: virtual lists details are only known by the server.

```
query("sex", "k=sex@", virtual = T)
sex$nelem
```

```
[1] 1080
```

There are 1,080 such sequences.

tRNA. How many complete tRNA sequences are available?

```
query("trna", "t=trna et no k=partial", virtual = T)
trna$nelem
```

```
[1] 179713
```

There are 179,713 complete tRNA sequences.

Nature vs. Science. In which journal were the more sequences published?

```
query("nature", "j=nature", virtual = T)
nature$nelem
```

```
[1] 1157543
```

```
query("science", "j=science", virtual = T)
science$nelem
```

```
[1] 1275773
```

There are 1,157,543 sequences published in *Nature* and 1,275,773 sequences published in *Science*, so that the winner is *Science*.

Smith. How many sequences have Smith (last name) as author?

```
query("smith", "au=smith", virtual = T)
smith$nelem
```

```
[1] 1393252
```

There are 1,393,252 such sequences.

YK2. How many sequences were published after year 2000 (included)?

```
query("yk2", "y>2000", virtual = T)
yk2$nelem
```

```
[1] 50248132
```

There are 50,248,132 sequences published after year 2000.

Organelle contest. Do we have more sequences from chloroplast genomes or from mitochondion genomes?

```
query("chloro", "o=chloroplast", virtual = T)
chloro$nelem
```

```
[1] 117315
```

```
query("mito", "o=mitochondrion", virtual = T)
mito$nelem
```

```
[1] 390956
```

There are 117,315 sequences from chloroplast genomes and 390,956 sequences from mitochondrion genomes, so that the winner is mitochondrion.

3.3 Extract sequences of interest

The sequence itself is obtained with the function `getSequence()`. For example, the first 50 nucleotides of the first sequence of our request are:

```
myseq <- getSequence(completeCatsCDS$req[[1]])
myseq[1:50]
```

```
 [1] "a" "t" "g" "a" "a" "t" "c" "a" "a" "g" "g" "a" "g" "c" "c" "g" "t" "t"
[19] "t" "t" "t" "a" "g" "g" "c" "a" "c" "c" "t" "g" "c" "t" "c" "c" "t" "g"
[37] "g" "t" "g" "c" "t" "g" "c" "a" "g" "c" "t" "g" "g" "t"
```

They can also be coerced as string of character with the function `c2s()`:

```
c2s(myseq[1:50])
```

```
[1] "atgaatcaaggagccgtttttaggcacctgctcctggtgctgcagctggt"
```

Note that what is done by `getSequence()` is much more complex than a substring extraction because subsequences of biological interest are not necessarily contiguous or even on the same DNA strand. Consider for instance the following coding sequence from sequence `AE003734`:

```
AE003734.PE35        Location/Qualifiers    (length=1833 bp)
     CDS             join(complement(162997..163210),
                     complement(162780..162919),complement(161238..162090),
                     146568..146732,146806..147266)
                     /gene="mod(mdg4)"
                     /locus_tag="CG32491"
                     /note="CG32491 gene product from transcript CG32491-RT;
                     trans-splicing"
```

To get the coding sequence manually you would have join 5 different pieces from `AE003734` and some of them are in the complementary strand. With `getSequence()` you don't have to think about this. Just make a query with the sequence name:

```
query("transspliced", "N=AE003734.PE35")
length(transspliced$req)
```

```
[1] 1
```

```
getName(transspliced$req[[1]])
```

```
[1] "AE003734.PE35"
```

Ok, now there is in your workspace an object called `transspliced` which `req` component is of length one (because you have asked for just one sequence) and the name of the single element of the req component is AE003734.PE35 (because this is the name of the sequence you wanted). Let see the first 50 base of this sequence:

```
getSequence(transspliced$req[[1]])[1:50]
```

```
 [1] "a" "t" "g" "g" "c" "g" "g" "a" "c" "g" "a" "c" "g" "a" "g" "c" "a" "a"
[19] "t" "t" "c" "a" "g" "c" "t" "t" "g" "t" "g" "c" "t" "g" "g" "a" "a" "c"
[37] "a" "a" "c" "t" "t" "c" "a" "a" "c" "a" "c" "g" "a" "a"
```

All the complex transsplicing operations have been done here. You can check that there is no in-frame stop codons[4] with the `getTrans()` function to translate this coding sequence into protein:

```
getTrans(transspliced$req[[1]])[1:50]
```

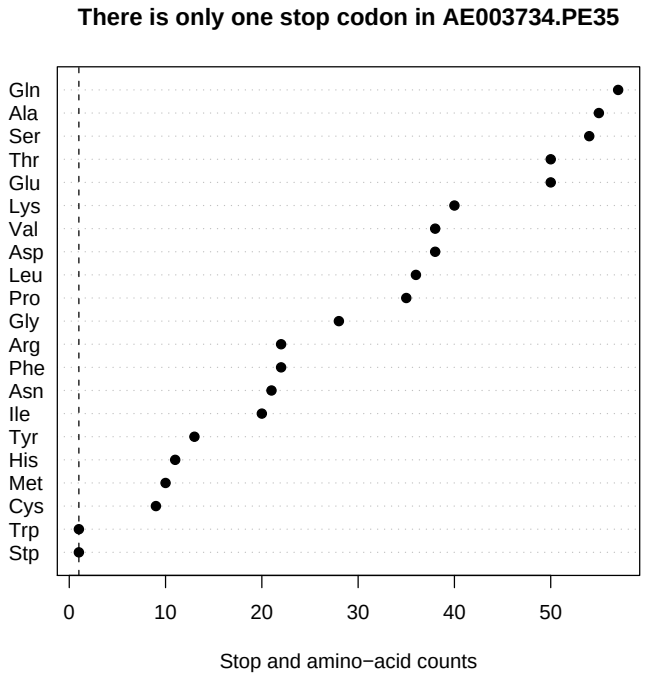
```
 [1] "M" "A" "D" "D" "E" "Q" "F" "S" "L" "C" "W" "N" "N" "F" "N" "T" "N" "L"
[19] "S" "A" "G" "F" "H" "E" "S" "L" "C" "R" "G" "D" "L" "V" "D" "V" "S" "L"
[37] "A" "A" "E" "G" "Q" "I" "V" "K" "A" "H" "R" "L" "V" "L"
```

```
table(getTrans(transspliced$req[[1]]))
```

```
*  A  C  D  E  F  G  H  I  K  L  M  N  P  Q  R  S  T  V  W  Y
1 55  9 38 50 22 28 11 20 40 36 10 21 35 57 22 54 50 38  1 13
```

In a more graphical way:

```
aacount <- table(getTrans(transspliced$req[[1]]))
aacount <- aacount[order(aacount)]
names(aacount) <- aaa(names(aacount))
dotchart(aacount, pch = 19, xlab = "Stop and amino-acid counts",
    main = "There is only one stop codon in AE003734.PE35")
abline(v = 1, lty = 2)
```



Note that the relevant variant of the genetic code was automatically set up during the translation of the sequence into protein. This is because the `transspliced$req[[1]]` object belongs to the `SeqAcnucWeb` class:

```
class(transspliced$req[[1]])
```

[4] Stop codons are represented by the character * when translated into protein.

```
[1] "SeqAcnucWeb"
```

Therefore, when you are using the `getTrans()` function, you are automatically redirected to the `getTrans.SeqAcnucWeb()` function which knows how to take into account the relevant frame and genetic code for your coding sequence.

3.4 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

CHAPTER 4

How to deal with sequences

Charif, D. Lobry, J.R.

Contents

4.1 Sequence classes

There are currently three classes of sequences, depending on the way they were obtained:

- **seqFasta** is the class for the sequences that were imported from a fasta file
- **seqAcnucWeb** is the class for the sequences coming from an ACNUC database server
- **seqFrag** is the class for the sequences that are fragments of other sequences

4.2 Generic methods for sequences

All sequence classes are sharing a common interface, so that there are very few method names we have to remember. In addition, all classes have their specific as.ClassName method that return an instance of the class, and is.ClassName method to check whether an object belongs or not to the class. Available methods are:

Methods	Result	Type of result
getFrag	a sequence fragment	a sequence fragment
getSequence	the sequence	vector of characters
getName	the name of a sequence	string
getLength	the length of a sequence	numeric vector
getTrans	translation into amino-acids	vector of characters
getAnnot	sequence annotations	vector of string
getLocation	position of a Sequence on its parent sequence	list of numeric vector

4.3 Internal representation of sequences

The current mode of sequence storage is done with vectors of characters instead of strings. This is very convenient for the user because all R tools to manipulate vectors are immediatly available. The price to pay is that this storage mode is extremly expensive in terms of memory. They are two utilities called `s2c()` and `c2s()` that allows to convert strings into vector of characters, and *vice versa*, respectively.

4.3.1 Sequences as vectors of characters

In the vectorial representation mode, all the very convenient R tools for indexing vectors are at hand.

1. Vectors can be indexed by a vector of *positive* integers saying which elements are to be selected. As we have already seen, the first 50 elements of a sequence are easily extracted thanks to the binary operator `from:to`, as in:

```
dnafile <- system.file("sequences/malM.fasta", package = "seqinr")
myseq <- read.fasta(file = dnafile)[[1]]
1:50
```

```
 [1]  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
[25] 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
[49] 49 50
```

```
myseq[1:50]
```

```
 [1] "a" "t" "g" "a" "a" "a" "a" "t" "g" "a" "a" "t" "a" "a" "a" "a" "g" "t"
[19] "c" "t" "c" "a" "t" "c" "g" "t" "c" "c" "t" "c" "t" "g" "t" "t" "t" "a"
[37] "t" "c" "a" "g" "c" "a" "g" "g" "g" "t" "t" "a" "c" "t"
```

   The `seq()` function allows to build more complexe integer vectors. For instance in coding sequences it is very common to focus on third codon positions where selection is weak. Let's extract bases from third codon positions:

```
tcp <- seq(from = 3, to = length(myseq), by = 3)
tcp[1:10]
```

```
 [1]  3  6  9 12 15 18 21 24 27 30
```

```
myseqtcp <- myseq[tcp]
myseqtcp[1:10]
```

```
[1] "g" "a" "g" "t" "a" "t" "c" "c" "c" "c"
```

2. Vectors can also be indexed by a vector of *negative* integers saying which elements have to be removed. For instance, if we want to keep first and second codon positions, the easiest way is to remove third codon positions:

```
-tcp[1:10]
```

```
[1]  -3  -6  -9 -12 -15 -18 -21 -24 -27 -30
```

```
myseqfscp <- myseq[-tcp]
myseqfscp[1:10]
```

```
[1] "a" "t" "a" "a" "a" "t" "a" "a" "a" "a"
```

3. Vectors are also indexable by a vector of *logicals* whose `TRUE` values say which elements to keep. Here is a different way to extract all third coding positions from our sequence. First, we define a vector of three logicals with only the last one true:

```
ind <- c(F, F, T)
ind
```

```
[1] FALSE FALSE  TRUE
```

This vector seems too short for our purpose because our sequence is much more longer with its 921 bases. But under R vectors are automatically *recycled* when they are not long enough:

```
(1:30)[ind]
```

```
[1]  3  6  9 12 15 18 21 24 27 30
```

```
myseqtcp2 <- myseq[ind]
```

The result should be the same as previously:

```
identical(myseqtcp, myseqtcp2)
```

```
[1] TRUE
```

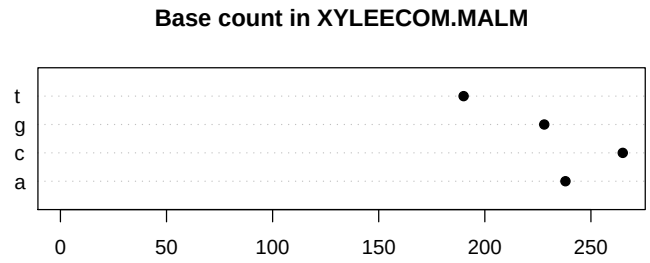
This recycling rule is extremely convenient in practice but may have surprising effects if you assume (incorrectly) that there is a stringent dimension control for R vectors as in linear algebra.

Another advantage of working with vector of characters is that most R functions are vectorized so that many things can be done without explicit looping. Let's give some very simple examples:

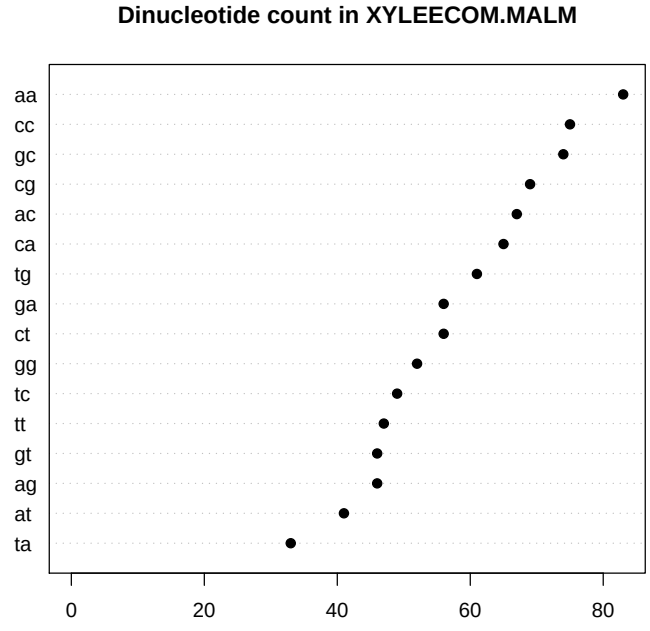
```
tota <- sum(myseq == "a")
```

The total number of `a` in our sequence is 238. Let's compare graphically the different base counts in our sequence :

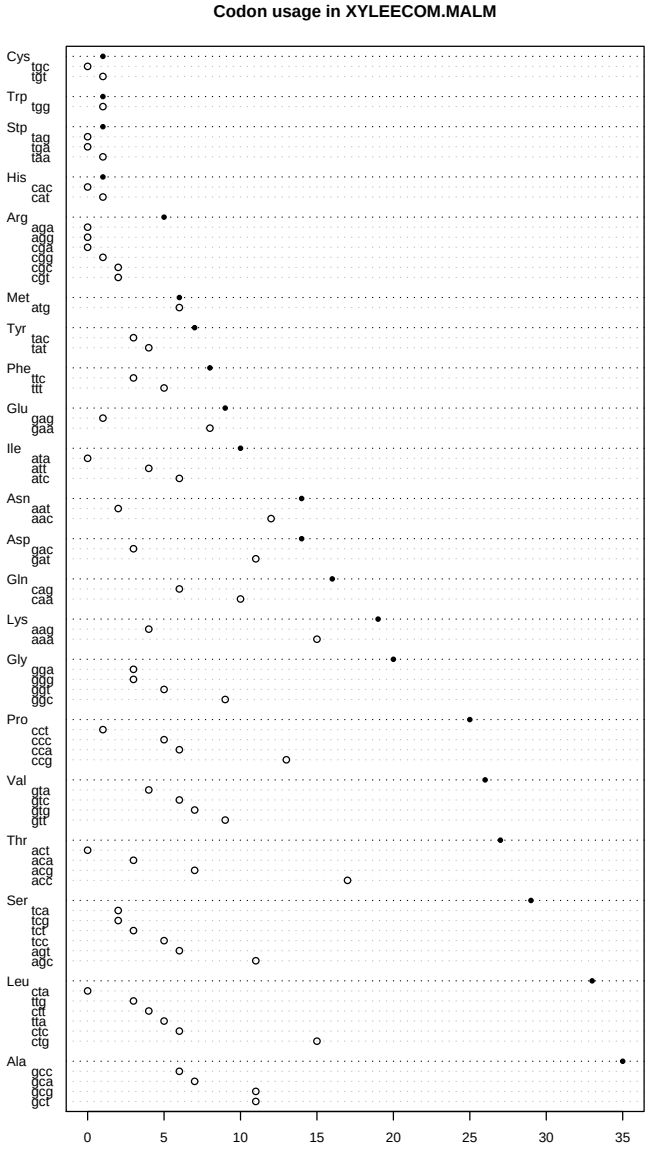
```
basecount <- table(myseq)
myseqname <- getName(myseq)
dotchart(basecount, xlim = c(0, max(basecount)), pch = 19,
    main = paste("Base count in", myseqname))
```




```
dinuclcount <- count(myseq, 2)
dotchart(dinuclcount[order(dinuclcount)], xlim = c(0, max(dinuclcount)),
    pch = 19, main = paste("Dinucleotide count in", myseqname))
```




```
codonusage <- uco(myseq)
dotchart.uco(codonusage, main = paste("Codon usage in", myseqname))
```



4.3.2 Sequences as strings

If you are interested in (fuzzy) pattern matching, then it is advisable to work with sequence as strings to take advantage of *regular expression* implemented in R. The function `words.pos()` returns the positions of all occurrences of a given regular expression. Let's suppose we want to know where are the trinucleotides "cgt" in a sequence, that is the fragment CpGpT in the direct strand:

```
mystring <- c2s(myseq)
words.pos("cgt", mystring)
```

```
[1]  24  90 216 245 252 315 330 405 432 452 552 592 648 836 883
```

We can also look for the fragment CpGpTpY to illustrate fuzzy matching because Y (IUPAC code for pyrimidine) stands C or T:

```
words.pos("cgt[ct]", mystring)
```

```
[1]  24 216 252 315 432 452 552 592 836 883
```

To look for all CpC dinucleotides separated by 3 or 4 bases:

```
words.pos("cc.{3,4}cc", mystring)
```

```
[1]  72 119 176 177 539 577 578 638 677 682 730 731 736 881 882
```

Virtually any pattern is easily encoded with a regular expression. This is especially useful at the protein level because many functions can be attributed to short linear motifs.

4.4 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

CHAPTER 5

Multivariate analyses

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Contents

5.1 Correspondence analysis

This is the most popular multivariate data analysis technique for amino-acid and codon count tables, its application, however, is not without pitfalls [45]. Its primary goal is to transform a table of counts into a graphical display, in which each gene (or protein) and each codon (or amino-acid) is depicted as a point. Correspondence analysis (CA) may be defined as a special case of principal components analysis (PCA) with a different underlying metrics. The interest of the metrics in CA, that is the way we measure the distance between two individuals, is illustrated bellow with a very simple example (Table 5.1 inspired from [10]) with only three proteins having only three amino-acids, so that we can represent exactly on a map the consequences of the metric choice.

```
data(toyaa)
toyaa
```

```
  Ala Val Cys
1 130  70   0
2  60  40   0
3  60  35   5
```

Let's first use the regular Euclidian metrics between two proteins i and i',

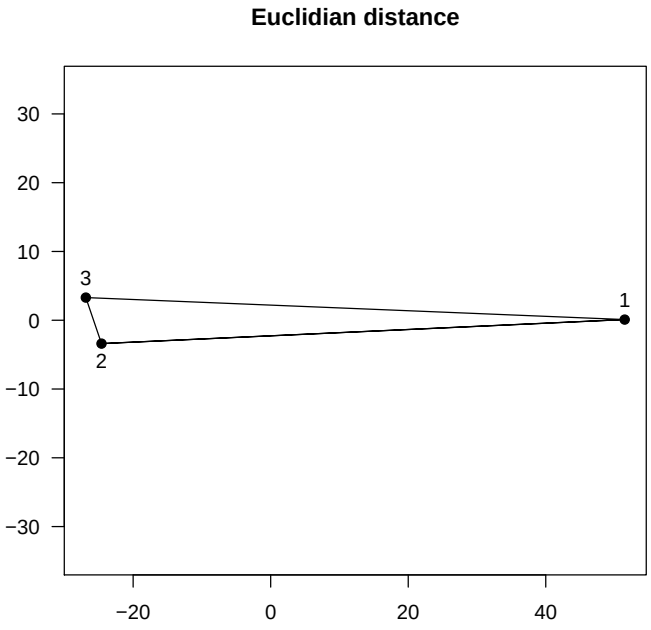
$$d^2(i,i') = \sum_{j=1}^{J}(n_{ij} - n_{i'j})^2 \tag{5.1}$$

	Ala	Val	Cys
1	130	70	0
2	60	40	0
3	60	35	5

Table 5.1: A very simple example of amino-acid counts in three proteins to be loaded with `data(toyaa)`.

to visualize this small data set:

```
library(ade4)
pco <- dudi.pco(dist(toyaa), scann = F, nf = 2)
myplot <- function(res, ...) {
    plot(res$li[, 1], res$li[, 2], ...)
    text(x = res$li[, 1], y = res$li[, 2], labels = 1:3, pos = ifelse(res$li[,
        2] < 0, 1, 3))
    perm <- c(3, 1, 2)
    lines(c(res$li[, 1], res$li[perm, 1]), c(res$li[, 2],
        res$li[perm, 2]))
}
myplot(pco, main = "Euclidian distance", asp = 1, pch = 19,
    xlab = "", ylab = "", las = 1)
```



From this point of view, the first individual is far away from the two others. But thinking about it, this is a rather trivial effect of protein size:

```
rowSums(toyaa)
```

```
  1   2   3
200 100 100
```

With 200 amino-acids, the first protein is two times bigger than the others so that when computing the Euclidian distance (5.1) its n_{ij} entries are on average bigger, sending it away from the others. To get rid of this trivial effect, the first obvious idea is to divide counts by protein lengths so as to work with *protein profiles*. The corresponding distance is,

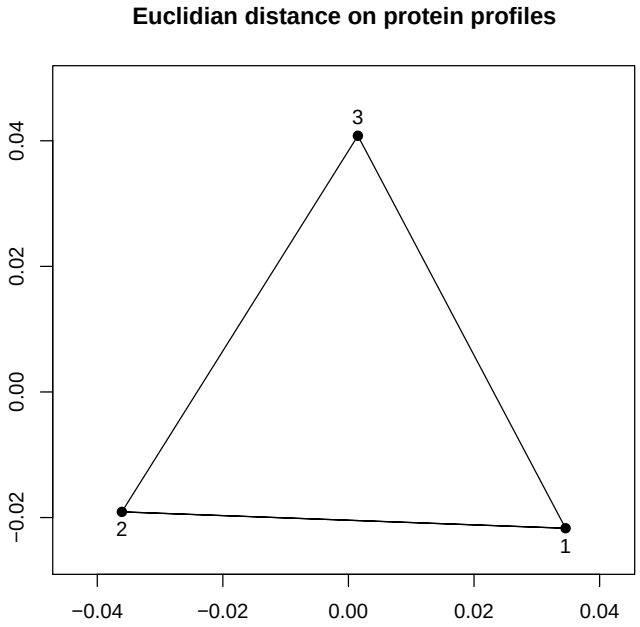
$$d^2(i,i') = \sum_{j=1}^{J} \left(\frac{n_{ij}}{n_{i\bullet}} - \frac{n_{i'j}}{n_{i'\bullet}}\right)^2 \tag{5.2}$$

where $n_{i\bullet}$ and $n_{i'\bullet}$ are the total number of amino-acids in protein i and i', respectively.

```
profile <- toyaa/rowSums(toyaa)
profile
```

```
   Ala  Val  Cys
1 0.65 0.35 0.00
2 0.60 0.40 0.00
3 0.60 0.35 0.05
```

```
pco1 <- dudi.pco(dist(profile), scann = F, nf = 2)
myplot(pco1, main = "Euclidian distance on protein profiles",
    asp = 1, pch = 19, xlab = "", ylab = "", ylim = range(pco1$li[,
        2]) * 1.2)
```



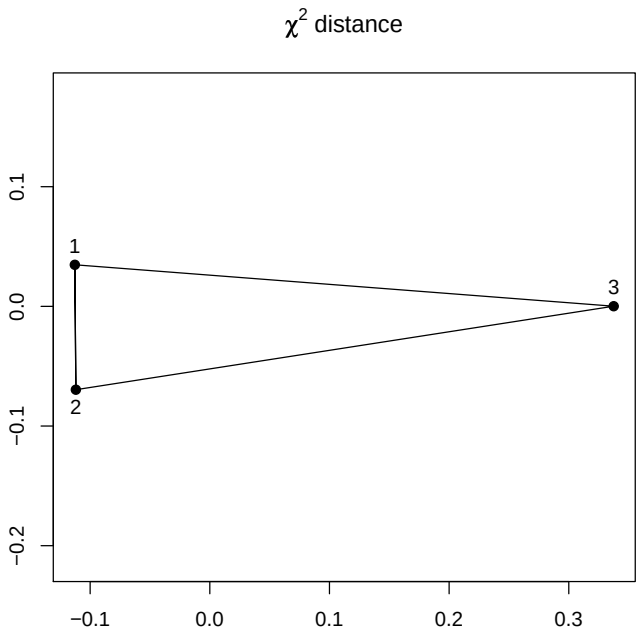
The pattern is now completely different with the three protein equally spaced. This is normal because in terms of relative amino-acid composition they are all differing two-by-two by 5% at the level of two amino-acids only. We have clearly removed the trivial protein size effect, but this is still not completely satisfactory. The proteins are differing by 5% for all amino-acids but the situation is

somewhat different for `Cys` because this amino-acid is very rare. A difference of 5% for a rare amino-acid has not the same significance than a difference of 5% for a common amino-acid such as `Ala` in our example. To cope with this, CA make use of a variance-standardizing technique to compensate for the larger variance in high frequencies and the smaller variance in low frequencies. This is achieved with the use of the *chi-square distance* (χ^2) which differs from the previous Euclidean distance on profiles (5.2) in that each square is weighted by the inverse of the frequency corresponding to each term,

$$d^2(i,i') = n_{\bullet\bullet} \sum_{j=1}^{J} \frac{1}{n_{\bullet j}} \left(\frac{n_{ij}}{n_{i\bullet}} - \frac{n_{i'j}}{n_{i'\bullet}}\right)^2 \tag{5.3}$$

where $n_{\bullet j}$ is the total number of amino-acid of kind j and $n_{\bullet\bullet}$ the total number of amino-acids. With this point of view, the map is now like this:

```
coa <- dudi.coa(toyaa, scann = FALSE, nf = 2)
myplot(coa, main = expression(paste(chi^2, " distance")),
    asp = 1, pch = 19, xlab = "", ylab = "")
```

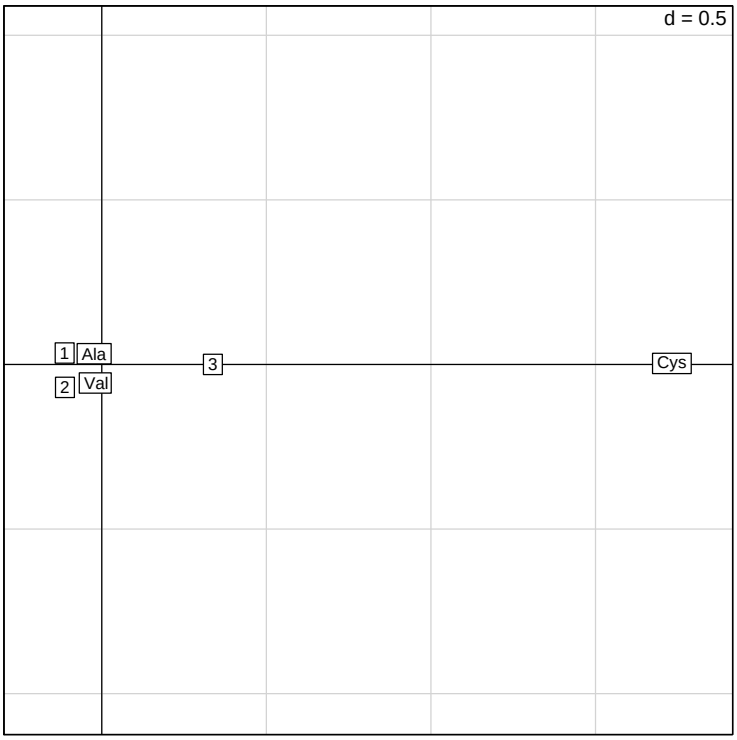


The pattern is completely different with now protein number 3 which is far away from the others because it is enriched in the rare amino-acid `Cys` as compared to others.

The purpose of this small example was to demonstrates that the metric choice is not without dramatic effects on the visualisation of data. Depending on your objectives, you may agree or disagree with the χ^2 metric choice, that's not a problem, the important point is that you should be aware that there is an underlying model there, *chacun a son goût* ou *chacun à son goût*, it's up to you.

Now, if you agree with the χ^2 metric choice, there's a nice representation that may help you for the interpretation of results. This is a kind of "biplot" representation in which the lines and columns of the dataset are simultaneously represented, in the right way, that is as a graphical *translation* of a mathematical theorem, but let's see how does it look like in practice:

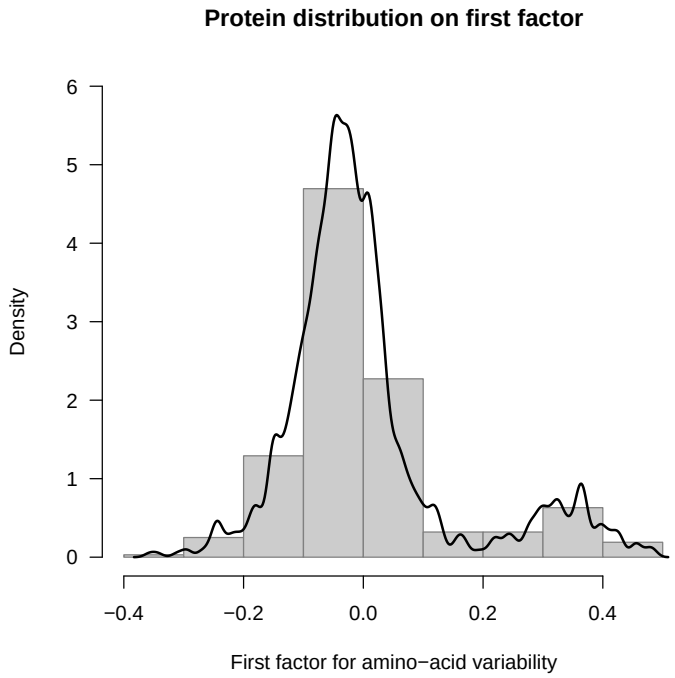
```
scatter(coa, clab.col = 0.8, clab.row = 0.8, posi = "none")
```



What is obvious is that the Cys content has a major effect on protein variability here, no scoop. Please note how the information is well summarised here: protein number 3 differs because it's enriched in in Cys ; protein number 1 and 2 are almost the same but there is a small trend protein number 1 to be enriched in Ala. As compared to to table 5.1 this graph is of poor information here, so let's try a more big-rooom-sized example (with 20 columns so as to illustrate the dimension reduction technique).

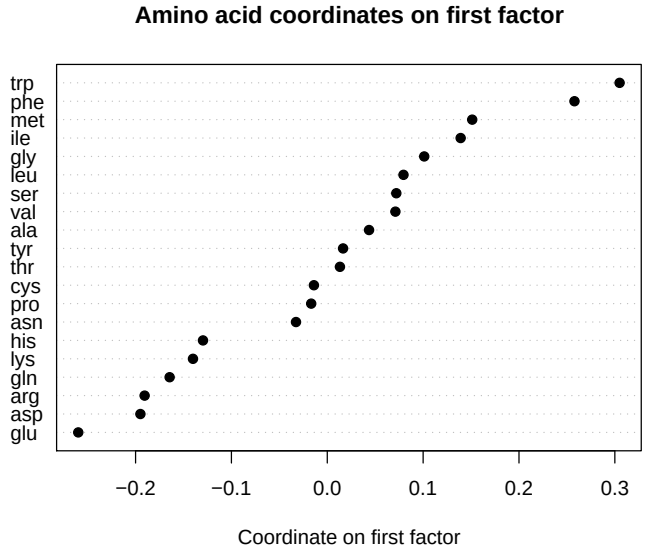
Data are from [35], a sample of the proteome of *Escherichia coli*. According to the title of this paper, the most important factor for the between-protein variability is hydrophilic - hydrophobic gradient. Let's try to reproduce this assertion :

```
download.file(url = "ftp://pbil.univ-lyon1.fr/pub/datasets/NAR94/data.txt",
    destfile = "data.txt")
ec <- read.table(file = "data.txt", header = TRUE, row.names = 1)
ec.coa <- dudi.coa(ec, scann = FALSE, nf = 1)
F1 <- ec.coa$li[, 1]
hist(F1, proba = TRUE, xlab = "First factor for amino-acid variability",
    col = grey(0.8), border = grey(0.5), las = 1, ylim = c(0,
        6), main = "Protein distribution on first factor")
lines(density(F1, adjust = 0.5), lwd = 2)
```



There is clearly a bimodal distribution of proteins on the first factor. What are the the amino-acid coordinates on this factor?

```
aacoo <- ec.coa$co[, 1]
names(aacoo) <- rownames(ec.coa$co)
aacoo <- sort(aacoo)
dotchart(aacoo, pch = 19, xlab = "Coordinate on first factor",
    main = "Amino acid coordinates on first factor")
```



Aliphatic and aromatic amino-acids have positive values while charged amino-acids have negative values[1]. Let's try to compute the GRAVY score (*i.e.* the Kyte and Doolittle hydropathic index[29]) of our proteins to compare this with their coordinates on the first factor. We need first the amino-acid *relatives* frequencies in the proteins, for this we divide the all the amino-acid counts by the total by row:

```
ecfr <- ec/rowSums(ec)
ecfr[1:5, 1:5]
```

```
            arg        leu        ser        thr        pro
FOLE 0.05829596 0.10313901 0.06278027 0.08520179 0.03587444
MSBA 0.06529210 0.10309278 0.08591065 0.06185567 0.02233677
NARV 0.06637168 0.12831858 0.06637168 0.05752212 0.03539823
NARW 0.05627706 0.16450216 0.05627706 0.03030303 0.04329004
NARY 0.06614786 0.06420233 0.05058366 0.03891051 0.06031128
```

We need also the coefficients corresponding to the GRAVY score:

```
gravy <- read.table(file = "ftp://pbil.univ-lyon1.fr/pub/datasets/NAR94/gravy.txt")
gravy[1:5, ]
```

```
   V1   V2
1 Ala  1.8
2 Arg -4.5
3 Asn -3.5
4 Asp -3.5
5 Cys  2.5
```

```
coef <- gravy$V2
```

The coefficient are given in the alphabetical order of the three letter code for the amino acids, that is in a different order than in the object `ecfr`:

```
names(ecfr)
```

```
 [1] "arg" "leu" "ser" "thr" "pro" "ala" "gly" "val" "lys" "asn" "gln" "his"
[13] "glu" "asp" "tyr" "cys" "phe" "ile" "met" "trp"
```

We then re-order the columns of the data set and check that everthing is OK:

```
ecfr <- ecfr[, order(names(ecfr))]
ecfr[1:5, 1:5]
```

```
            ala        arg        asn        asp         cys
FOLE 0.08520179 0.05829596 0.04035874 0.05381166 0.008968610
MSBA 0.08247423 0.06529210 0.03608247 0.05154639 0.003436426
NARV 0.05309735 0.06637168 0.01769912 0.02212389 0.013274336
NARW 0.09090909 0.05627706 0.02597403 0.09090909 0.017316017
NARY 0.06225681 0.06614786 0.03891051 0.05642023 0.035019455
```

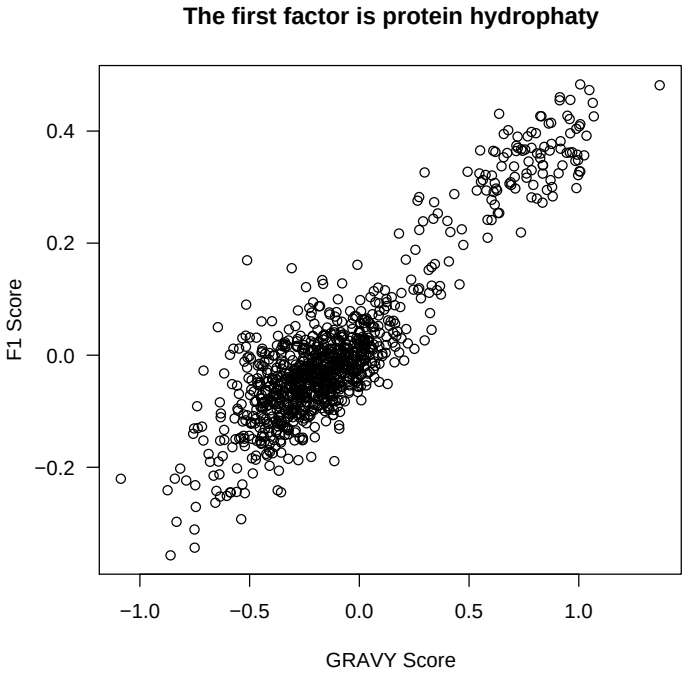
```
all(names(ecfr) == tolower(as.character(gravy$V1)))
```

```
[1] TRUE
```

[1] The physico-chemical classes for amino acids are given in the component `AA.PROPERTY` of the `SEQINR.UTIL` object.

Now, thanks to R build-in matrix multiplication, it's only one line to compute the GRAVY score:

```
gscores <- as.matrix(ecfr) %*% coef
plot(gscores, F1, xlab = "GRAVY Score", ylab = "F1 Score",
    las = 1, main = "The first factor is protein hydrophaty")
```



The proteins with high GRAVY scores are integral membrane proteins, and those with low scores are cytoplasmic proteins. Now, suppose that we want to adjust a mixture of two normal distributions to get an estimate of the proportion of cytoplasmic and integral membrane proteins. We first have a look on the predefined distributions (Table 5.2), but there is apparently not an out of the box solution. We then define our own probability density function and then use `fitdistr` from package `MASS` to get a maximum likelihood estimate of the parameters:

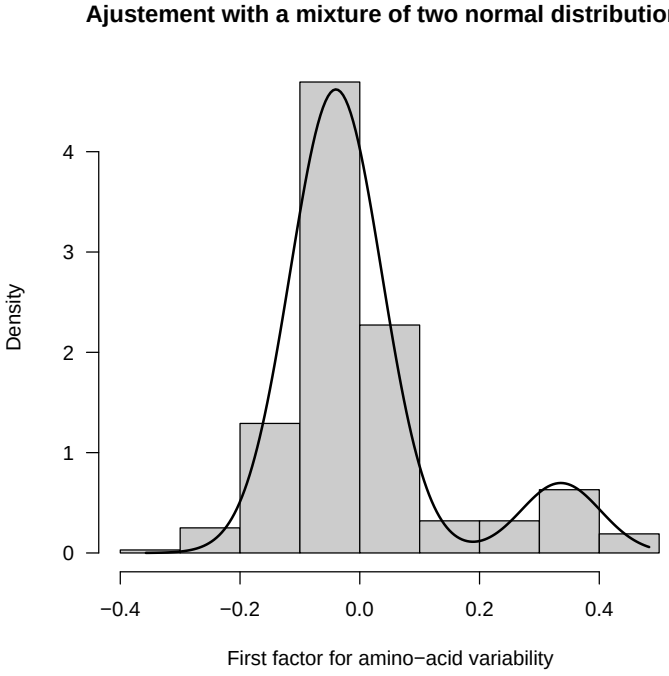
```
dmixnor <- function(x, p, m1, sd1, m2, sd2) {
    p * dnorm(x, m1, sd1) + (1 - p) * dnorm(x, m2, sd2)
}
library(MASS)
e <- fitdistr(F1, dmixnor, list(p = 0.88, m1 = -0.04, sd1 = 0.076,
    m2 = 0.34, sd2 = 0.07))$estimate
e
```

```
         p          m1         sd1          m2         sd2
0.88405009 -0.03989489  0.07632235  0.33579162  0.06632259
```

```
hist(F1, proba = TRUE, col = grey(0.8), main = "Ajustement with a mixture of two normal distributions",
    xlab = "First factor for amino-acid variability", las = 1)
xx <- seq(from = min(F1), to = max(F1), length = 200)
lines(xx, dmixnor(xx, e[1], e[2], e[3], e[4], e[5]), lwd = 2)
```

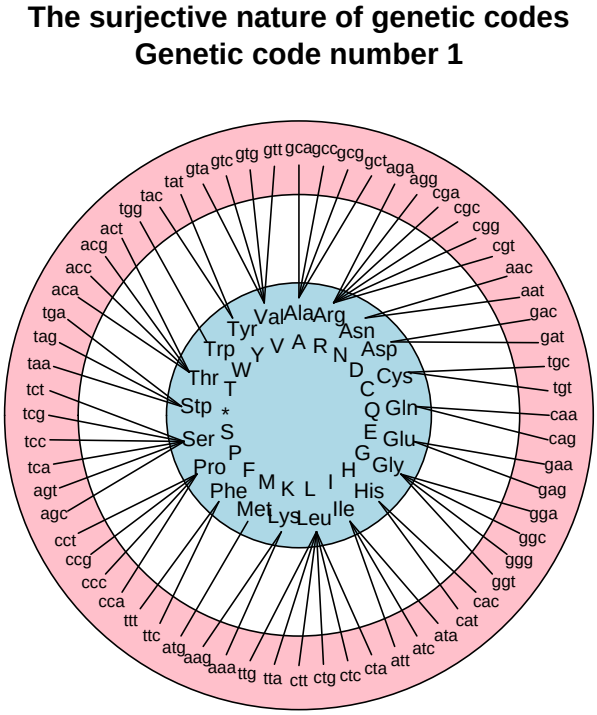
	d	p	q	r
beta	dbeta	pbeta	qbeta	rbeta
binom	dbinom	pbinom	qbinom	rbinom
cauchy	dcauchy	pcauchy	qcauchy	rcauchy
chisq	dchisq	pchisq	qchisq	rchisq
exp	dexp	pexp	qexp	rexp
f	df	pf	qf	rf
gamma	dgamma	pgamma	qgamma	rgamma
geom	dgeom	pgeom	qgeom	rgeom
hyper	dhyper	phyper	qhyper	rhyper
lnorm	dlnorm	plnorm	qlnorm	rlnorm
logis	dlogis	plogis	qlogis	rlogis
nbinom	dnbinom	pnbinom	qnbinom	rnbinom
norm	dnorm	pnorm	qnorm	rnorm
pois	dpois	ppois	qpois	rpois
signrank	dsignrank	psignrank	qsignrank	rsignrank
t	dt	pt	qt	rt
unif	dunif	punif	qunif	runif
weibull	dweibull	pweibull	qweibull	rweibull
wilcox	dwilcox	pwilcox	qwilcox	rwilcox

Table 5.2: Density, distribution function, quantile function and random generation for the predefined distributions under R



5.2 Synonymous and non-synonymous analyses

Genetic codes are surjective applications from the set codons ($n = 64$) into the set of amino-acids ($n = 20$) :



Adapted from insert 2 in Lobry & Chessel (2003) JAG 44:235

Two codons encoding the same amino-acid are said synonymous while two codons encoding a different amino-acid are said non-synonymous. The distinction between the synonymous and non-synonymous level are very important in evolutionary studies because most of the selective pressure is expected to work at the non-synonymous level, because the amino-acids are the components of the proteins, and therefore more likely to be subject to selection.

K_s and K_a are an estimation of the number of substitutions per synonymous site and per non-synonymous site, respectively, between two protein-coding genes [32]. The $\frac{K_a}{K_s}$ ratio is used as tool to evaluate selective pressure (see [20] for a nice back to basics). Let's give a simple illustration with three orthologous genes of the thioredoxin familiy from *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus* species:

```
ortho <- read.alignment(system.file("sequences/ortho.fasta",
    package = "seqinr"), format = "fasta")
kaks.ortho <- kaks(ortho)
kaks.ortho$ka/kaks.ortho$ks
```

```
             AK002358.PE1 HSU78678.PE1
HSU78678.PE1    0.1243472
RNU73525.PE1    0.1405012    0.1356036
```

The $\frac{K_a}{K_s}$ ratios are less than 1, suggesting a selective pressure on those proteins during evolution.

For transversal studies (*i.e.* codon usage studies in a genome at the time it was sequenced) there is little doubt that the strong requirement to distinguish between synonymous and an non-synonymous variability was the source of many mistakes [45]. We have just shown here with a scholarship example that the metric choice is not neutral. If you consider that the χ^2 metric is not too bad, with respect to your objectives, and that you want to quantify the synonymous and an non-synonymous variability, please consider reading this paper [34], and follow this link `http://pbil.univ-lyon1.fr/members/lobry/repro/jag03/` for on-line reproducibility.

Let's now use the toy example given in table 5.3 to illustrate how to study synonymous and non-synonymous codon usage.

```
data(toycodon)
toycodon
```

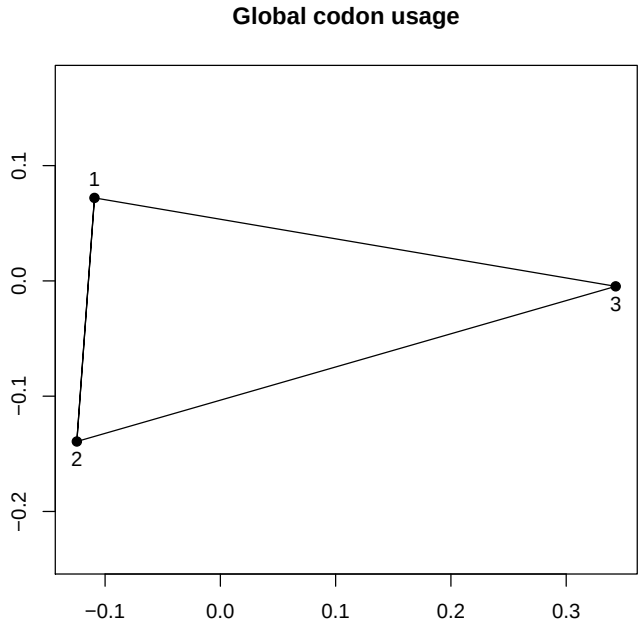
```
  gca gcc gcg gct gta gtc gtg gtt tgt tgc
1  33  32  32  33  18  17  17  18   0   0
2  13  17  17  13   8  12  12   8   0   0
3  16  14  14  16   8   9  10   8   3   2
```

	gca	gcc	gcg	gct	gta	gtc	gtg	gtt	tgt	tgc
1	33	32	32	33	18	17	17	18	0	0
2	13	17	17	13	8	12	12	8	0	0
3	16	14	14	16	8	9	10	8	3	2

Table 5.3: A very simple example of codon counts in three coding sequences to be loaded with `data(toycodon)`.

Let's first have a look to global codon usage, we do not take into account the structure of the genetic code:

```
global <- dudi.coa(toycodon, scann = FALSE, nf = 2)
myplot(global, asp = 1, pch = 19, xlab = "", ylab = "", main = "Global codon usage")
```



From a global codon usage point of view, coding sequence number 3 is away. To take into account the genetic code structure, we need to know for which amino-acid the codons are coding. The codons are given by the names of the columns of the object `toycodon`:

```
names(toycodon)
```

```
 [1] "gca" "gcc" "gcg" "gct" "gta" "gtc" "gtg" "gtt" "tgt" "tgc"
```

Put all codon names into a single string:

```
c2s(names(toycodon))
```

```
[1] "gcagccgcggctgtagtcgtggtttgttgc"
```

Transform this string as a vector of characters:

```
s2c(c2s(names(toycodon)))
```

```
 [1] "g" "c" "a" "g" "c" "c" "g" "c" "g" "g" "c" "t" "g" "t" "a" "g" "t" "c"
[19] "g" "t" "g" "g" "t" "t" "t" "g" "t" "t" "g" "c"
```

Translate this into amino-acids using the default genetic code:

```
translate(s2c(c2s(names(toycodon))))
```

```
 [1] "A" "A" "A" "A" "V" "V" "V" "V" "C" "C"
```

Use the three letter code for amino-acid instead:

```
aaa(translate(s2c(c2s(names(toycodon)))))
```

```
 [1] "Ala" "Ala" "Ala" "Ala" "Val" "Val" "Val" "Val" "Cys" "Cys"
```

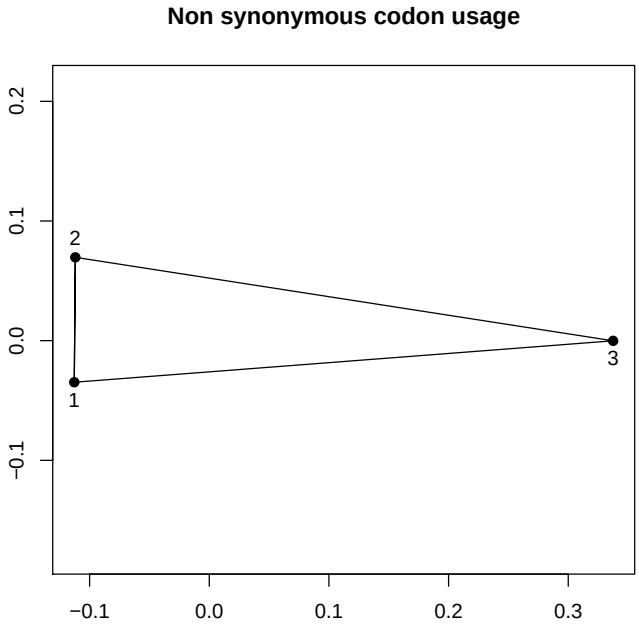
Make this a factor:

```
facaa <- factor(aaa(translate(s2c(c2s(names(toycodon))))))
facaa
```

```
 [1] Ala Ala Ala Ala Val Val Val Val Cys Cys
Levels: Ala Cys Val
```

The non synonymous codon usage analysis is the between amino-acid analysis:

```
nonsynonymous <- t(between(dudi = t(global), fac = facaa,
    scann = FALSE, nf = 2))
myplot(nonsynonymous, asp = 1, pch = 19, xlab = "", ylab = "",
    main = "Non synonymous codon usage")
```



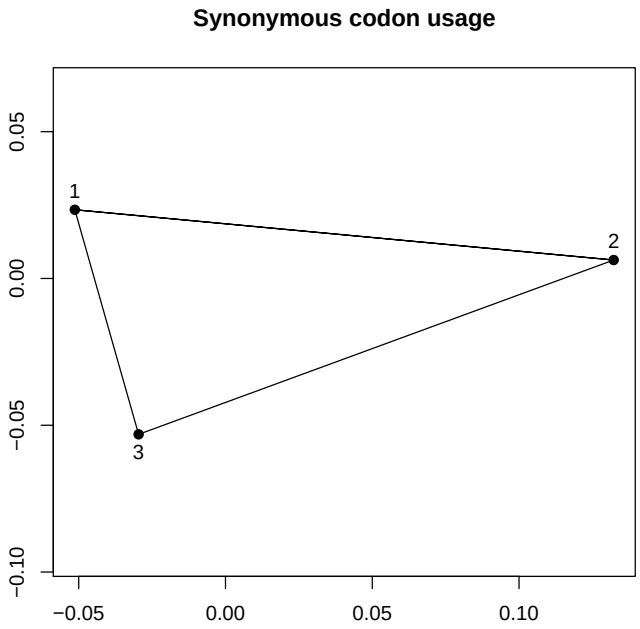
This is reminiscent of something, let's have a look at amino-acid counts:

```
by(t(toycodon), facaa, colSums)
```

```
INDICES: Ala
  1   2   3
130  60  60
------------------------------------------------------------
INDICES: Cys
1 2 3
0 0 5
------------------------------------------------------------
INDICES: Val
 1  2  3
70 40 35
```

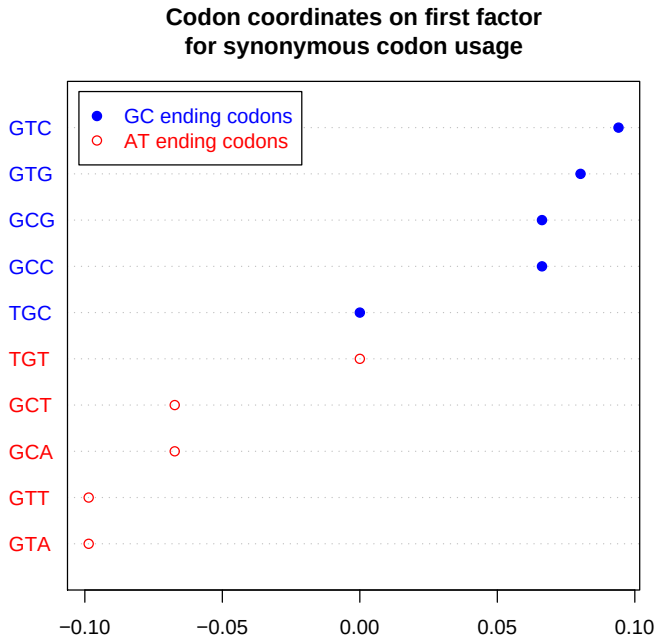
This is exactly the same data set that we used previously (table 5.1) at the amino-acid level. The non synonymous codon usage analysis is exactly the same as the amino-acid analysis. Coding sequence number 3 is far away because it codes for many Cys, a rare amino-acid. Note that at the global codon usage level, this is also the major visible structure. To get rid of this amino-acid effect, we use the synonymous codon usage analysis, that is the within amino-acid analysis:

```
synonymous <- t(within(dudi = t(global), fac = facaa, scann = FALSE,
    nf = 2))
myplot(synonymous, asp = 1, pch = 19, xlab = "", ylab = "",
    main = "Synonymous codon usage")
```



Now, coding sequence number 2 is away. When the amino-acid effect is removed, the pattern is then completely different. To interpret the result we look at the codon coordinates on the first factor of synonymous codon usage:

```
tmp <- synonymous$co[, 1, drop = FALSE]
tmp <- tmp[order(tmp$Axis1), , drop = FALSE]
colcod <- sapply(rownames(tmp), function(x) ifelse(substr(x,
    3, 3) == "c" || substr(x, 3, 3) == "g", "blue", "red"))
pchcod <- ifelse(colcod == "red", 1, 19)
dotchart(tmp$Axis1, labels = toupper(rownames(tmp)), color = colcod,
    pch = pchcod, main = "Codon coordinates on first factor\nfor synonymous codon usage")
legend("topleft", inset = 0.02, legend = c("GC ending codons",
    "AT ending codons"), text.col = c("blue", "red"), pch = c(19,
    1), col = c("blue", "red"), bg = "white")
```



At the synonymous level, coding sequence number 2 is different because it is enriched in GC-ending codons as compared to the two others. Note that this is hard to see at the global codon usage level because of the strong amino-acid effect.

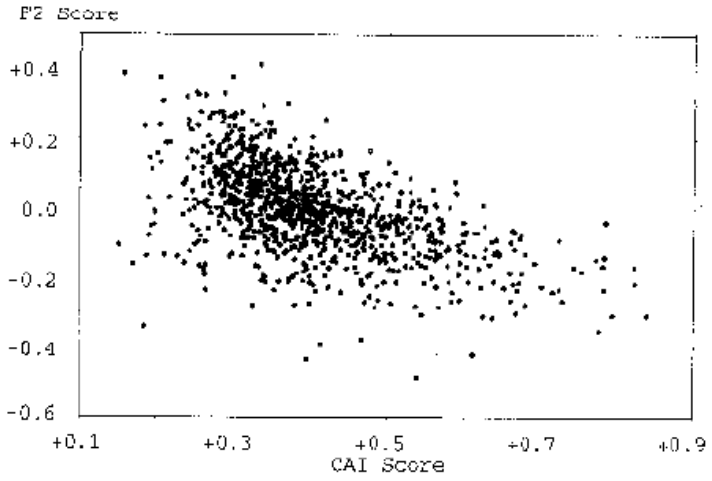
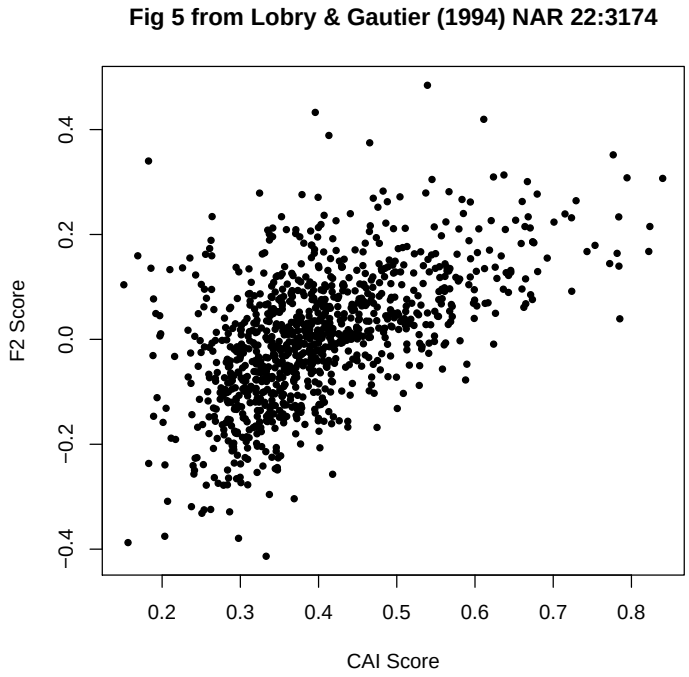


Figure 5.1: Screenshot of figure 5 from [35]. Each point represents a protein. This was to show the correlation between the codon adaptation index (CAI Score) with the second factor of correspondence analysis at the amino-acid level (F2 Score). Highly expressed genes have a high CAI value.

To illustrate the interest of synonymous codon usage analyses, let's use now a more realistic example. In [35] there was an assertion stating that selection for translation optimisation in *Escherichia coli* was also visible at the amino-acid level. The argument was in figure 5 of the paper (*cf* fig 5.1), that can be reproduced[2] with the following R code:

```
ec <- read.table(file = "ftp://pbil.univ-lyon1.fr/pub/datasets/NAR94/data.txt",
    header = TRUE, row.names = 1)
ec.coa <- dudi.coa(ec, scann = FALSE, nf = 3)
tmp <- read.table(file = "ftp://pbil.univ-lyon1.fr/pub/datasets/NAR94/ecoli999.cai")
cai <- exp(tmp$V2)
plot(cai, ec.coa$li[, 2], pch = 20, xlab = "CAI Score", ylab = "F2 Score",
    main = "Fig 5 from Lobry & Gautier (1994) NAR 22:3174")
```



So, there was a correlation between the CAI (Codon Adaptation Index [51]) and the second factor for amino-acid composition variability. However, this is not completely convincing because the CAI is not completely independent of the amino-acid composition of the protein. Let's use within amino-acid correspondence analysis to remove the amino-acid effect. Here is a commented step-by-step analysis:

```
data(ec999)
class(ec999)
```

```
[1] "list"
```

```
names(ec999)[1:10]
```

[2] the code to reproduce all figures from [35] is available at http://pbil.univ-lyon1.fr/members/lobry/repro/nar94/.

```
 [1] "ECFOLE.FOLE"     "ECMSBAG.MSBA"    "ECNARZYW-C.NARV" "ECNARZYW-C.NARW"
 [5] "ECNARZYW-C.NARY" "ECNARZYW-C.NARZ" "ECNIRBC.NIRB"    "ECNIRBC.NIRD"
 [9] "ECNIRBC.NIRC"    "ECNIRBC.CYSG"
```

```
ec999[[1]][1:50]
```

```
 [1] "a" "t" "g" "c" "c" "a" "t" "c" "a" "c" "t" "c" "a" "g" "t" "a" "a" "a"
[19] "g" "a" "a" "g" "c" "g" "g" "c" "c" "c" "t" "g" "g" "t" "t" "c" "a" "t"
[37] "g" "a" "a" "g" "c" "g" "t" "t" "a" "g" "t" "t" "g" "c"
```

This is to load the data from [35] which is available as `ec999` in the **seqinR** package. The letters `ec` are for the bacterium *Escherichia coli* and the number `999` means that there were 999 coding sequences available from this species at that time. The class of the object `ec999` is a list, which names are the coding sequence names, for instance the first coding sequence name is `ECFOLE.FOLE`. Each element of the list is a vector of character, we have listed just above the 50 first character of the first coding sequence of the list with `ec999[[1]][1:50]`, we can see that there is a start codon (ATG) at the beginning of the first coding sequence.

```
ec999.uco <- lapply(ec999, uco)
class(ec999.uco)
```

```
[1] "list"
```

```
class(ec999.uco[[1]])
```

```
[1] "table"
```

```
ec999.uco[[1]]
```

```
aaa aac aag aat aca acc acg act aga agc agg agt ata atc atg att caa cac cag
  9   5   2   4   2   8   8   1   0   2   0   4   0   9   8   6   2   3   7
cat cca ccc ccg cct cga cgc cgg cgt cta ctc ctg ctt gaa gac gag gat gca gcc
  7   1   1   6   0   1   7   1   4   1   3  13   3  12   3   1   9   1   6
gcg gct gga ggc ggg ggt gta gtc gtg gtt taa tac tag tat tca tcc tcg tct tga
  7   5   2   3   0   4   0   5   9   4   0   2   0   2   2   3   2   1   1
tgc tgg tgt tta ttc ttg ttt
  1   0   1   1   4   2   3
```

This is to compute the codon usage, that is how many times each codon is used in each coding sequence. Because `ec999` is a list, we use the function `lapply()` to apply the same function, `uco()`, to all the elements of the list and we store the result in the object `ec999.uco`. The object `ec999.uco` is a list too, and all its elements belong to the class table.

```
df <- as.data.frame(lapply(ec999.uco, as.vector))
dim(df)
```

```
[1]  64 999
```

```
df[1:5, 1:5]
```

```
  ECFOLE.FOLE ECMSBAG.MSBA ECNARZYW.C.NARV ECNARZYW.C.NARW ECNARZYW.C.NARY
1           9           15               2               6              23
2           5           18               2               4              16
3           2            8               1               3               4
4           4            3               2               2               4
5           2            3               1               1               0
```

This is to put the codon usage into a data.frame. Note that the codons are in row and the coding sequences are in columns. This is more convenient for the following because groups for within and between analyses are usually handled by row.

```
row.names(df) <- names(ec999.uco[[1]])
df[1:5, 1:5]
```

```
    ECFOLE.FOLE ECMSBAG.MSBA ECNARZYW.C.NARV ECNARZYW.C.NARW ECNARZYW.C.NARY
aaa           9           15               2               6              23
aac           5           18               2               4              16
aag           2            8               1               3               4
aat           4            3               2               2               4
aca           2            3               1               1               0
```

This is to keep a trace of codon names, just in case we would like to re-order the dataframe `df`. This is important because we can now play with the data at will without loosing any critical information.

```
ec999.coa <- dudi.coa(df = df, scannf = FALSE)
ec999.coa
```

```
Duality diagramm
class: coa dudi
$call: dudi.coa(df = df, scannf = FALSE)

$nf: 2 axis-components saved
$rank: 63
eigen values: 0.05536 0.02712 0.02033 0.01884 0.01285 ...
  vector length mode    content
1 $cw    999    numeric column weights
2 $lw    64     numeric row weights
3 $eig   63     numeric eigen values

  data.frame nrow ncol content
1 $tab       64   999  modified array
2 $li        64   2    row coordinates
3 $l1        64   2    row normed scores
4 $co        999  2    column coordinates
5 $c1        999  2    column normed scores
other elements: N
```

This is to run global correspondence analysis of codon usage. We have set the `scannf` parameter to `FALSE` because otherwise the eigenvalue bar plot is displayed for the user to select manually the number of axes to be kept.

```
facaa <- as.factor(aaa(translate(s2c(c2s(rownames(df))))))
facaa
```

```
 [1] Lys Asn Lys Asn Thr Thr Thr Thr Arg Ser Arg Ser Ile Ile Met Ile Gln His
[19] Gln His Pro Pro Pro Pro Arg Arg Arg Arg Leu Leu Leu Leu Glu Asp Glu Asp
[37] Ala Ala Ala Ala Gly Gly Gly Gly Val Val Val Val Stp Tyr Stp Tyr Ser Ser
[55] Ser Ser Stp Cys Trp Cys Leu Phe Leu Phe
21 Levels: Ala Arg Asn Asp Cys Gln Glu Gly His Ile Leu Lys Met Phe ... Val
```

This is to define a factor for amino-acids. The function `translate()` use by default the standard genetic code and this is OK for *E. coli*.

```
ec999.syn <- within(dudi = ec999.coa, fac = facaa, scannf = FALSE)
ec999.syn
```

```
Within analysis
call: within(dudi = ec999.coa, fac = facaa, scannf = FALSE)
class: within dudi

$nf (axis saved) : 2
$rank:  43
$ratio:  0.6438642

eigen values: 0.04855 0.0231 0.01425 0.007785 0.006748 ...

  vector length mode    content
1 $eig   43     numeric eigen values
2 $lw    64     numeric row weigths
3 $cw    999    numeric col weigths
4 $tabw  21     numeric table weigths
5 $fac   64     numeric factor for grouping

  data.frame nrow ncol content
1 $tab       64   999  array class-variables
2 $li        64   2    row coordinates
3 $l1        64   2    row normed scores
4 $co        999  2    column coordinates
5 $c1        999  2    column normed scores
6 $ls        64   2    supplementary row coordinates
7 $as        2    2    inertia axis onto within axis
```

This is to run the synonymous codon usage analysis. The value of the `ratio` component of the object `ec999.syn` shows that most of the variability is at the synonymous level, a common situation in codon usage studies.

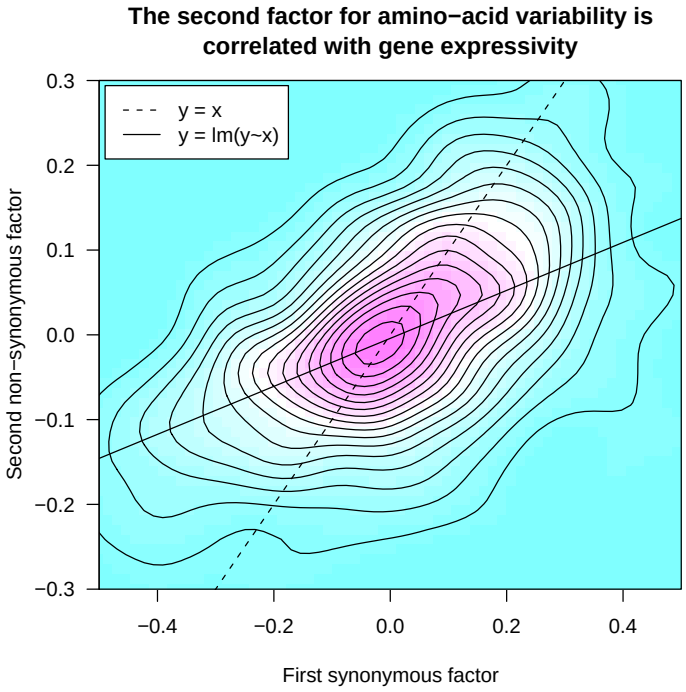
```
ec999.btw <- between(dudi = ec999.coa, fac = facaa, scannf = FALSE)
ec999.btw
```

```
Between analysis
call: between(dudi = ec999.coa, fac = facaa, scannf = FALSE)
class: between dudi

$nf (axis saved) : 2
$rank:  20
$ratio:  0.3561358

eigen values: 0.01859 0.0152 0.01173 0.01051 0.008227 ...

  vector length mode    content
1 $eig   20     numeric eigen values
2 $lw    21     numeric group weigths
3 $cw    999    numeric col weigths

  data.frame nrow ncol content
1 $tab       21   999  array class-variables
2 $li        21   2    class coordinates
3 $l1        21   2    class normed scores
4 $co        999  2    column coordinates
5 $c1        999  2    column normed scores
6 $ls        64   2    row coordinates
7 $as        2    2    inertia axis onto between axis
```

This is to run the non-sysnonymous codon usage analysis, or amino-acid usage analysis.

```
x <- ec999.syn$co[, 1]
y <- ec999.btw$co[, 2]
kxy <- kde2d(x, y, n = 100)
nlevels <- 25
breaks <- seq(from = min(kxy$z), to = max(kxy$z), length = nlevels +
    1)
col <- cm.colors(nlevels)
image(kxy, breaks = breaks, col = col, xlab = "First synonymous factor",
    ylab = "Second non-synonymous factor", xlim = c(-0.5,
```

```
        0.5), ylim = c(-0.3, 0.3), las = 1, main = "The second factor for amino-acid variability is\ncor
contour(kxy, add = TRUE, nlevels = nlevels, drawlabels = FALSE)
box()
abline(c(0, 1), lty = 2)
abline(lm(y ~ x))
legend("topleft", lty = c(2, 1), legend = c("y = x", "y = lm(y~x)"),
    inset = 0.01, bg = "white")
```



This is to plot the whole thing. We have extracted the coding sequences coordinates on the first synonymous factor and the second non-synonymous factor within `x` and `y`, respectively. Because we have many points, we use the two-dimensional kernel density estimation provided by the function `kde2d()` from package `MASS`.

To be completed

5.3 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

	aaa	a	prec	p	h	tot	gc
1	Ala	A	pyr	1	5	12	h
2	Cys	C	3pg	7	9	25	m
3	Asp	D	oaa	1	6	13	m
4	Glu	E	akg	3	6	15	m
5	Phe	F	2 pep, eryP	13	19	52	l
6	Gly	G	3pg	2	5	12	h
7	His	H	penP	20	9	38	m
8	Ile	I	pyr, oaa	4	14	32	l
9	Lys	K	oaa, pyr	4	13	30	l
10	Leu	L	2 pyr, acCoA	3	12	27	l
11	Met	M	oaa, Cys, -pyr	10	12	34	m
12	Asn	N	oaa	3	6	15	l
13	Pro	P	akg	4	8	20	h
14	Gln	Q	akg	4	6	16	m
15	Arg	R	akg	11	8	27	h
16	Ser	S	3pg	2	5	12	m
17	Thr	T	oaa	3	8	19	m
18	Val	V	2 pyr	2	11	23	m
19	Trp	W	2 pep, eryP, PRPP, -pyr	28	23	74	m
20	Tyr	Y	eryP, 2 pep	13	18	50	l

Table 5.4: Aerobic cost of amino-acids in *Escherichia coli* and G+C classes to be loaded with `data(aacost)`.

CHAPTER 6

Nonparametric statistics

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Contents

6.1 Introduction

Nonparametric statistical methods were initially developped to study variables for which little or nothing is known concerning their distribution. This makes them particularly suitable for statistical analysis of biological sequences, in particular for the study of over- and under-representation of k-letter words.

We will briefly describe two statistics for the measure of dinucleotide over- and under-representation in sequences [25, 42], which can both be computed with `seqin`**R**. We will subsequently use them to answer the long-time controversial question concerning the relationship between UV exposure and genomic content in bacteria [52, 1].

6.2 Determining dinucleotides over- and under-representation

6.2.1 The *rho* statistic

The ρ statistic (`rho()`), presented in [25], measures the over- and under-representation of two-letter words:

$$\rho(xy) = \frac{f_{xy}}{f_x \times f_y}$$

where f_{xy} and f_x are respectively the frequencies of dinucleotide xy and nucleotide x in the studied sequence. The underlying model of random generation considers dinucleotides to be formed according to the specific frequencies of the two nucleotides that compose it ($\rho_{xy} = 1$). Departure from this value characterizes either over- or under-representation of dinucleotide xy.

We expect the ρ statistic of a randomly generated sequence to be neither over- nor under-represented. Indeed, when we compute the ρ statistic on 500 random sequences, we can fit a normal distribution which is centered on 1 (see Fig. 6.1)

```
rhoseq <- sapply(seq(n), function(x) {
    rho(sample(s2c("acgt"), 6000, rep = TRUE))
})
hist(rhoseq[di, ], freq = FALSE, xlab = "Rho statistic", main = paste("Distribution for dinucleotide",
    toupper(labels(rhoseq)[[1]][di]), "on", n, "random sequences"),
    las = 1, col = grey(0.8), border = grey(0.5))
abline(v = 1, lwd = 2, lty = 3)
min = min(rhoseq[di, ])
max = max(rhoseq[di, ])
x = seq(min, max, length.out = 1000)
lines(x, dnorm(x, mean = mean(rhoseq[di, ]), sd = sd(rhoseq[di,
    ])), lwd = 2, lty = 2)
```

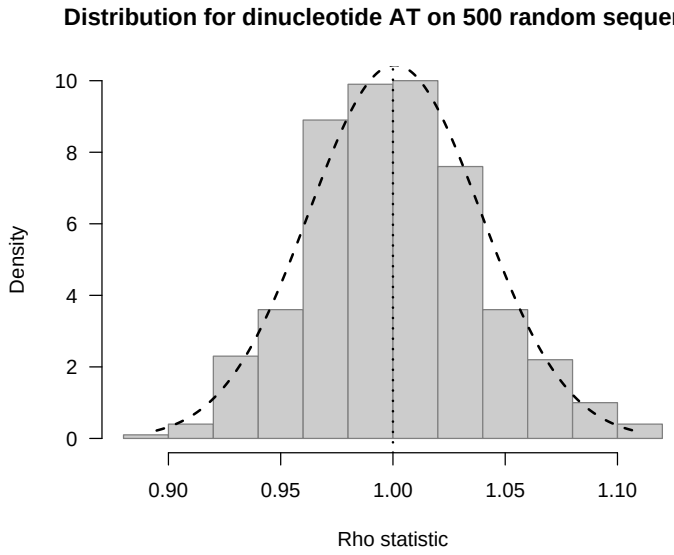


Figure 6.1: Distribution of the ρ statistic computed on 500 random sequences. The vertical dotted line is centered on 1. The dashed curve draws the fitted normal distribution.

The downside of this statistic, is that the model against which we compare the sequence under study is fixed. For several types of sequences, dinucleotides

are far from being formed by mere chance (CDS, ...). In this case, the model used in the ρ statistic becomes trivial, and the over- or under-representations measured are mainly due to the strong constraints acting on those sequences.

6.2.2 The *zscore* statistic

The *zscore* statistic (`zscore()`) is inspired by the ρ statistic, and is defined so that several different models can be used for the determination of over- and under-representation [42]. It allows for a finer measure of over- and under-representation in sequences, according to the chosen model.

The *zscore* is defined as follows:

$$z_{score} = \frac{\rho_{xy} - E(\rho_{xy})}{\sqrt{Var(\rho_{xy})}}$$

where $E(\rho_{xy})$ and $Var(\rho_{xy})$ are the expected mean and variance of ρ_{xy} according to a given model that describes the sequence.

This statistic follows the standard normal distribution, and can be computed with several different models of random sequence generation based on permutations from the original sequence (`modele` argument). More details on those models can be obtained in the documentation for the `zscore()` function, by simply typing `?zscore`.

For instance, if we want to measure the over- and under-representation of dinucleotides in CDS sequences, we can use the `codon` model, which measures the over- and under-representations existing in the studied sequence once codon usage bias has been erased. For intergenic sequences, or sequences for which no good permutation model can be established, we can use the `base` model.

6.2.3 Comparing statistics on a sequence

Let's have a look at what these different statistics can show. First, we will extract a CDS sequence of *Escherichia coli*'s chromosome from the Genome Reviews database. We will first make a request to retrieve all available CDS from this bacteria:

```
choosebank("greview")
query("coli", "sp=escherichia coli et t=cds et no k=partial")
sequence <- getSequence(coli$req[[448]])
```

From the 3684 sequences annotated as CDS in the Genome Reviews database, let's choose one coding sequence: say, for instance, number `448`. We can see that this CDS encodes a maltose O-acetyltransferase protein (`getAnnot(coli$req[[448]],30)`). We will now compare the three following nonparametric statistics:

- the ρ statistic,
- the *zscore* statistic with `base` model,
- and the *zscore* statistic with `codon` model.

```
rhocoli = rho(sequence)
zcolibase = zscore(sequence, mod = "base")
zcolicodon = zscore(sequence, mod = "codon")
par(mfrow = c(1, 3))
plot(rhocoli - 1, ylim = c(-0.5, 0.5), las = 1, ylab = "rho")
plot(zcolibase, ylim = c(-2.5, 2.5), las = 1, ylab = "zscore with base model")
plot(zcolicodon, ylim = c(-2.5, 2.5), las = 1, ylab = "zscore with codon model")
```

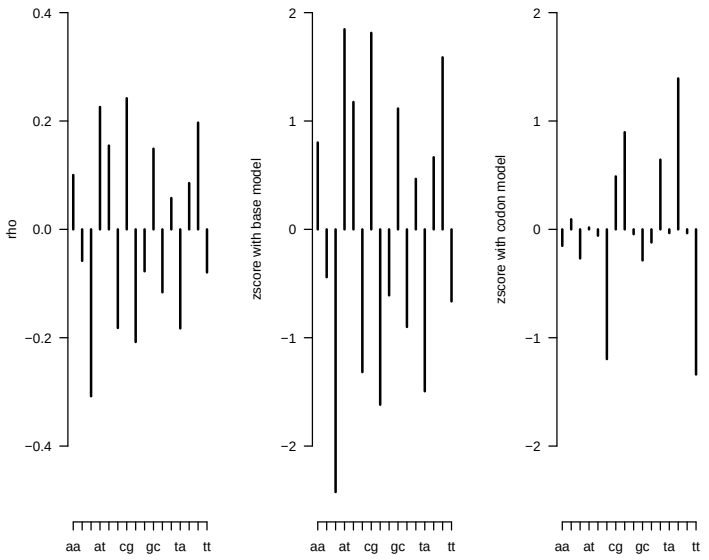


Figure 6.2: Three different non-parametric statistics (from left to right: ρ, *zscore* with `base` model, *zscore* with `codon` model), computed on the same sequence from *Escherichia coli*. In order to make the figures easily comparable, we substracted 1 to the `rho()` results, so that all 3 statistics are centered on 0.

The first two figures are almost identical: this is due to the way the *zscore* statistic has been built. The statistic computed with the `base` model is a reflection of the ρ statistic. The difference being that the *zscore* follows a standard normal distribution, which makes easier the comparisons between the results from the `base` model and the ones from the `codon` model. The last figure (*zscore* with `codon` model), is completely different: almost all over- and under-representations have been erased. We can safely say that these over- and under-representations were due to codon usage bias.

On this last figure, four dinucleotides stand out: CC and TT seem rather under-represented, CT and TC rather over-represented. This means that, in this sequence, codons ending with a given pyrimidine tend to be more frequently followed by a codon starting with the other pyrimidine than expected by chance. This is not a universal feature of *Escherichia coli*, and is probably due to the amino-acid composition of this particular sequence. It seemed a funny example,

as the following part will also relate to pyrimidine dinucleotides. However, what we see on this CDS from *Escherichia coli* has nothing to do with what follows...

6.3 UV exposure and dinucleotide content

In the beginning of the 1970's, two contradictory papers considered the question of the impact of UV exposure on genomic content. Both papers had strong arguments for either side, and the question remained open until recently [42].

6.3.1 The expected impact of UV light on genomic content

On this controversy, the known facts are: pyrimidine dinucleotides (CC, TT, CT and TC) are the major DNA target for UV-light [50]; the sensitivities of the four pyrimidine dinucleotides to UV wavelengths differ and depend on the micro-organism [50]:

	G+C content	CC (%)	CT + TC (%)	TT (%)
Haemophilus influenzae	62	5	24	71
Escherichia coli	50	7	34	59
Micrococcus lysodeikticus	30	26	55	19

Table 6.1: Proportion of dimers formed in the DNA of three bacteria after irradiation with 265 nm UV light. Table adapted from [50].

The hypothesis presented by Singer and Ames [52] is that pyrimidine dinucleotides are avoided in light-exposed micro-organisms. At the time, only G+C content is available, and – based exclusively on the sensitivity of the four pyrimidine dinucleotides in an *Escherichia coli* chromosome – they hypothesize that a high G+C will result in less pyrimidine target. Indeed, they find that bacteria exposed to high levels of UV have higher G+C content than the others. Bak *et al.* [1] strongly criticize their methodology, but no clear cut answer is achieved.

In an *Escherichia coli* chromosome, it is true that a sequence with a high G+C content will contain few phototargets (see Fig. 6.3).

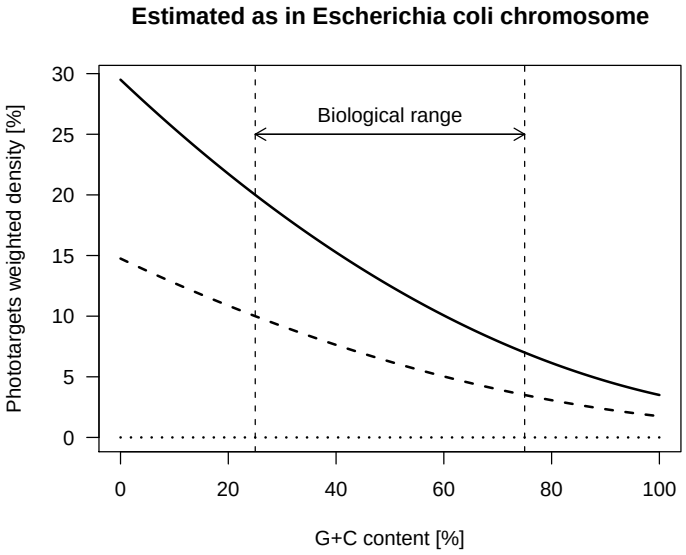


Figure 6.3: Density of phototargets, weighted by their frequency in the *Escherichia coli* chromosome, and calculated for different G+C contents and for three kinds of random genomes. The weights are as follows: $0.59 * f_{tt} + 0.34 * (f_{tc} + f_{ct}) + 0.07 * f_{cc}$ (where f_{xy} is the frequency of dinucleotide xy in the specified genome). Three models of random genomes are analyzed. In the worst case (solid curve), the genome is the concatenation of a sequence of pyrimidines and a sequence of purines: all pyrimidines are involved in a pyrimidine dinucleotide. In the best case (dotted curve), the genome is an unbroken succession of pyrimidine-purine dinucleotides: no pyrimidine is involved in a pyrimidine dinucleotide. In the "random case" (dashed curve), the frequency of a pyrimidine dinucleotide is the result of chance ($f_{xy} = f_x \times f_y$).

In a *Micrococcus lysodeikticus* sequence (see Fig. 6.4), we can see that this is no longer true...

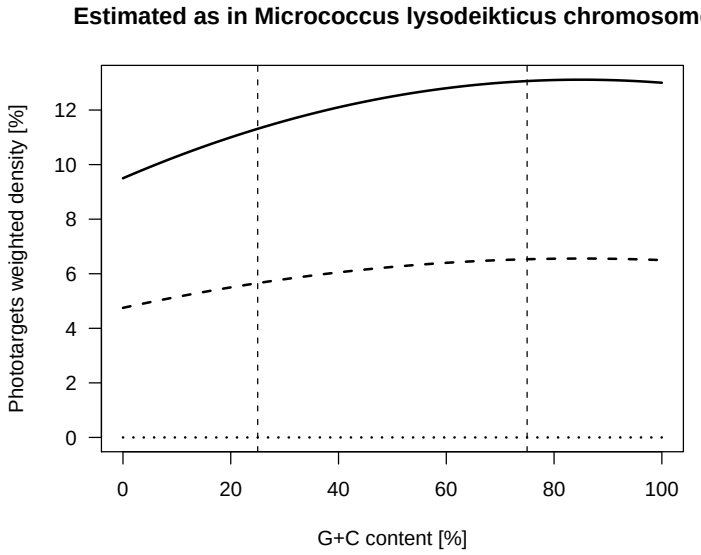


Figure 6.4: Density of phototargets, weighted by their frequency in the *Micrococcus lysodeikticus* chromosome, and calculated for different G+C contents and for three kinds of random genomes. The weights are as follows: $0.19 * f_{tt} + 0.55 * (f_{tc} + f_{ct}) + 0.26 * f_{cc}$. See previous figure for more details.

These two figures show that the density of phototargets depends on:

- the degree of aggregation of pyrimidine dinucleotides in the sequence,
- the sensitivities of the four pyrimidine dinucleotides.

Instead of looking at G+C content, which is an indirect measure of the impact of UV exposure on genomic content, let us look at pyrimidine dinucleotide content.

Are CC, TT, CT and TC dinucleotides avoided in light-exposed bacteria?

6.3.2 The measured impact of UV light on genomic content

On all available genomes (as retrieved from Genome Reviews database on June 16, 2005), we have computed the mean of the *zscore* with the `base` model on all intergenic sequences, and the mean of the *zscore* with the `codon` model on all CDS:

```
data(dinucl)
```

The results show that there is no systematic under-representation of none of the four pyrimidine dinucleotides (see Fig. 6.5).

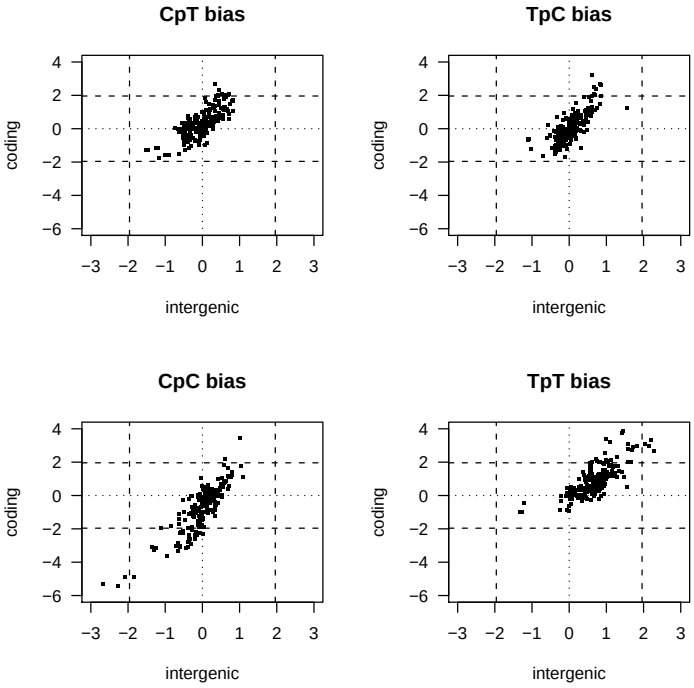


Figure 6.5: Plot of the mean *zscore* statistics for **intergenic sequences** (x-axis) and for **coding sequences** (y-axis), for each of the four pyrimidine dinucleotides. On each plot, a dot corresponds to the mean of these two statistics in a given prokaryote chromosome. The null x and y axis (dotted lines), and the 5% limits of significance for the standard normal distribution (dashed lines) are plotted as benchmarks. It should be noted that the variability within one chromosome is sometimes as great as that between different chromosomes.

However, we have little or no information on the exposure of this bacteria to UV light. In order to fully answer this question, let's do another analysis and look at *Prochlorococcus marinus* genome.

Prochlorococcus marinus seems to make an ideal model for investigating this hypothesis. Three completely sequenced strains are available in the Genome reviews database: two of these strains are adpated to living at a depth of more than 120 meters (accession numbers AE017126 and BX548175), and the other one at a depth of 5 meters (accession number BX548174).

Living at a depth of 5 meters, or at a depth of more than a 120 meters is totally different in terms of UV exposure: the residual intensity of 290 nm irradiation (UVb) in pure water can be estimated to 56% of its original intensity at 5 m depth and to less than 0.0001% at more than 120 m depth. For this reason, two of the *Prochlorococcus marinus* strains can be considered to be adapted to low levels of UV exposure, and the other one to much higher levels. Is pyrimidine dinucleotide content different in these three strains? And is it linked to their UV exposure?

We have computed the *zscore* with the `codon` model on all CDS from each of these three strains (as retrieved from Genome Reviews database on June 16, 2005):

```
data(prochlo)
```

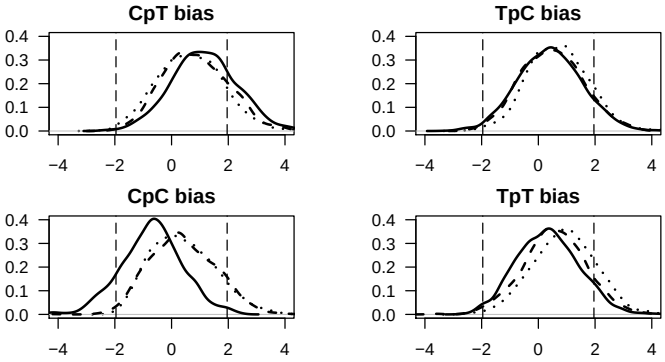


Figure 6.6: Each figure shows the distributions of the *zscore* in all **coding sequences** corresponding to each of the three strains of *Prochlorococcus marinus*. In each figure, the distribution for the MED4 (a high-light adapted strain) is shown as a solid line; the distribution for the SS120 (a low-light adapted strain) is shown as a dashed line, and the distribution for the MIT 9313 (a low-light adapted strain) is shown as a dotted line. The 5% limits of significance for the standard normal distribution (dashed vertical lines) are plotted as benchmarks.

Figure 6.6 shows that there is no difference between the relative abundances of pyrimidine dinucleotides in these three strains. We can say that pyrimidine dinucleotides are not avoided, and that the hypothesis by Singer and Ames [52] no longer stands [42].

6.4 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

APPENDIX A

FAQ: Frequently Asked Questions

Lobry, J.R.

A.1 How do I compute a score on my sequences?

In the example below we want to compute the G+C content in third codon positions for complete ribosomal CDS from *Escherichia coli*:

```
choosebank("emblTP")
query("ecribo", "sp=escherichia coli ET t=cds ET k=ribosom@ ET NO k=partial")
myseqs <- sapply(ecribo$req, getSequence)
(gc3 <- sapply(myseqs, GC3))
```

```
 [1] 0.4946237 0.6046512 0.5000000 0.6194030 0.5772727 0.4838710 0.5980066
 [8] 0.4974359 0.5031250 0.4324324 0.5000000 0.5113636 0.5290520 0.6142857
[15] 0.4904762 0.5714286 0.6191860 0.5906040 0.4880000 0.4880000 0.4946237
[22] 0.6046512 0.5000000 0.3522727 0.5076923 0.4343434 0.6194030 0.5522388
[29] 0.6104651 0.5661157 0.4946237 0.4946237 0.6079734 0.5000000 0.6343284
[36] 0.4659091 0.5789474 0.4946237 0.5000000 0.4974359 0.5689655 0.4611111
[43] 0.4611111 0.5303030 0.5303030 0.4482759 0.4201681 0.5915493 0.5000000
[50] 0.3829787 0.4519231 0.4302326 0.5696203 0.4285714 0.5689655 0.5000000
[57] 0.5224417 0.5661157 0.6057692 0.4444444 0.4659091 0.4130435 0.4946237
[64] 0.5661157 0.4946237 0.5680272
```

At the amino-acid level, we may get an estimate of the isoelectric point of the proteins this way:

```
sapply(sapply(myseqs, getTrans), computePI)
```

```
 [1]  6.624309  7.801329 10.864793  5.931989  7.830476  6.624309  7.801329
 [8]  9.203410  9.826485  5.674672  7.154423  6.060457  6.313741  5.571446
[15]  9.435422  4.310747  6.145496  4.876054 11.006424 10.876041  6.624309
[22]  7.801329 10.864793  9.346289  9.203410  5.877050  5.931989  9.934988
[29]  5.920490  6.612505  6.624309  6.624309  7.801329 10.864793  5.931989
[36] 11.182505  9.598944  6.624309 10.864793  9.203410 11.031938  5.858421
[43]  5.858421 11.777516 11.777511 10.619175 11.365738  9.460987 10.864793
[50] 13.002381  9.845859 10.584868 11.421257 10.248325 11.031938 10.402075
[57]  4.863862  6.612505  9.681066 11.150304 11.182505 11.043602  6.624309
[64]  6.612505  6.624309  4.310747
```

Note that some pre-defined vectors to compute linear forms on sequences are available in the `EXP` data.

As a matter of convenience, you may encapsulate the computation of your favorite score within a function this way:

```
GC3m <- function(list, ind = 1:list$nelem) sapply(sapply(list$req[ind],
    getSequence), GC3)
GC3m(ecribo)
```

```
 [1] 0.4946237 0.6046512 0.5000000 0.6194030 0.5772727 0.4838710 0.5980066
 [8] 0.4974359 0.5031250 0.4324324 0.5000000 0.5113636 0.5290520 0.6142857
[15] 0.4904762 0.5714286 0.6191860 0.5906040 0.4880000 0.4880000 0.4946237
[22] 0.6046512 0.5000000 0.3522727 0.5076923 0.4343434 0.6194030 0.5522388
[29] 0.6104651 0.5661157 0.4946237 0.4946237 0.6079734 0.5000000 0.6343284
[36] 0.4659091 0.5789474 0.4946237 0.5000000 0.4974359 0.5689655 0.4611111
[43] 0.4611111 0.5303030 0.5303030 0.4482759 0.4201681 0.5915493 0.5000000
[50] 0.3829787 0.4519231 0.4302326 0.5696203 0.4285714 0.5689655 0.5000000
[57] 0.5224417 0.5661157 0.6057692 0.4444444 0.4659091 0.4130435 0.4946237
[64] 0.5661157 0.4946237 0.5680272
```

```
GC3m(ecribo, 1:10)
```

```
 [1] 0.4946237 0.6046512 0.5000000 0.6194030 0.5772727 0.4838710 0.5980066
 [8] 0.4974359 0.5031250 0.4324324
```

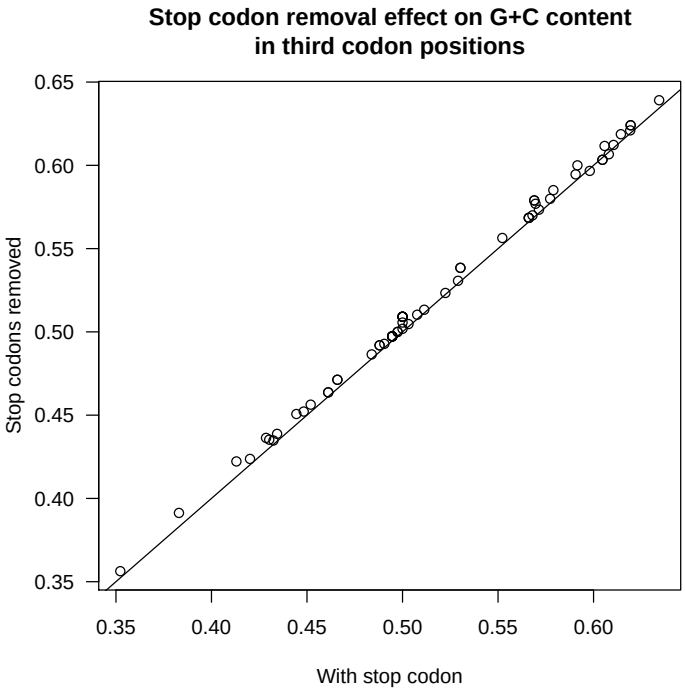
A.2 Why do I have not exactly the same G+C content as in `codonW`?

This question was raised (and solved) by Oliver Clay in an e-mail (23-AUG-2006). The program `codonW` was written in C as part of Jonh Peden's PhD thesis on Codon Usage [44] and is available at `http://codonw.sourceforge.net/`. The reason for the small differences in G+C content between the two programs is that the default behavior in `codonW` is to remove the stop codon before computations. Here is one way of removing the stop codon under R:

```
gc3nos <- sapply(myseqs, function(s) GC3(s[1:(length(s) -
    3)]))
```

As compared with the previous result, the difference is small but visible:

```
plot(x = gc3, y = gc3nos, las = 1, main = "Stop codon removal effect on G+C content\nin third codon posi
    xlab = "With stop codon", ylab = "Stop codons removed")
abline(c(0, 1))
```



CodonW was released with a test file called input.dat, here are the first 10 lines of the file copied from CodonWSourceCode_1_4_4:

```
inputdatfile <- system.file("sequences/input.dat", package = "seqinr")
cat(readLines(inputdatfile, n = 10), sep = "\n")
```

```
>YCG9 Probable          1377 residues Pha 0 Code 0
ATGAATATGCTCATTGTCGGTAGAGTTGTTGCTAGTGTTGGGGGAAGCGGACTTCAAACG
CTTTGCTTTGTTATTGGTTGTACGATGGTTGGTGAAAGGTCACGTCCATTGGTGATTTCC
ATCCTAAGTTGTGCATTTGCTGTAGCTGCTATCGTTGGTCCTATAATCGGAGGTGCCTTT
ACAACCCATGTTACCTGGAGGTGGTGCTTCTATATCAATCTTCCTATCGGTGGTCTTGCC
ATTATTATGTTTTTACTCACATATAAGGCCGAGAATAAGGGTATACTTCAACAAATTAAA
GATGCTATAGGAACAATCTCGAGCTTTACTTTTAGTAAGTTCAGACACCAAGTTAATTTT
AAAAGACTTATGAATGGCATAATCTTCAAGTTTGACTTCTTTGGTTTTGCCCTCTGCTCT
GCAGGGCTGGTCCTTTTCCTACTGGGGCTAACCTTTGGTGGTAATAAATATAGTTGGAAC
TCTGGCCAAGTCATCGCATATTTGGTTTTGGGTGTCTTACTTTTTATTTTTTCATTGGTG
```

This is a FASTA file that we import under R with:

```
input <- read.fasta(file = inputdatfile)
names(input)
```

```
  [1] "YCG9"      "YCG8"      "ALPHA2"    "ALPHA1"    "CHA1"      "KRR1"
  [7] "PRD1"      "KAR4"      "PBN1"      "LRE1"      "APA1"      "YCE9"
 [13] "YCE8"      "YCE7"      "YCE5"      "YCE6"      "YCE4"      "PDI1"
 [19] "GLK1"      "YCD8"      "SRO9"      "YCD6"      "YCD5"      "YCD3"
 [25] "STE50"     "HIS4"      "BIK1"      "FUS1"      "YCO8"      "AGP1"
 [31] "LEU2"      "NFS1"      "BUD3"      "GBP2"      "ILV6"      "CWH36"
 [37] "PEL1"      "RER1"      "CDC10"     "MRPL32"    "YCP4"      "CIT2"
 [43] "YCP7"      "SAT4"      "RVS161"    "YCQ0"      "ADP1"      "PGK1"
 [49] "POL4"      "YCQ7"      "SRD1"      "MAK32"     "PET18"     "MAK31"
 [55] "HSP30"     "YCR3"      "SYN"       "YCR6"      "GNS1"      "FEN2"
 [61] "RIM1"      "CRY1"      "YCS2"      "YCS3"      "GNS1"      "RBK1"
 [67] "PHO87"     "BUD5"      "MATALPHA2" "MATALPHA1" "TSM1"      "YCT5"
 [73] "PETCR46"   "YCT7"      "YCT9"      "ARE1"      "RSC6"      "THR4"
 [79] "CTR86"     "PWP2"      "YCU9"      "YCV1"      "G10"       "HCM1"
 [85] "RAD18"     "CYPR"      "YCW1"      "YCW2"      "SSK22"     "SOL2"
 [91] "ERS1"      "PAT1"      "SRB8"      "YCX3"      "TUP1"      "YC16"
 [97] "ABP1"      "KIN82"     "MSH3"      "CDC39"     "YCY4"      "A2"
[103] "GIT1"      "YCZ0"      "YCZ1"      "YCZ2"      "YCZ3"      "PAU3"
[109] "YCZ5"      "YCZ6"      "YCZ7"
```

The file `input.out` contains the values obtained with `codonW` for the GC content and GC3s content:

```
inputoutfile <- system.file("sequences/input.out", package = "seqinr")
cat(readLines(inputoutfile, n = 10), sep = "\n")
```

```
title                          GC3s        GC
YCG9_Probable_____________13   0.335        0.394
YCG8_________573_residues_     0.439        0.446
ALPHA2_________633_residue     0.328        0.351
ALPHA1_________528_residue     0.345        0.379
CHA1___________1083_residue    0.328        0.394
KRR1___________951_residue     0.364        0.384
PRD1___________2139_residue    0.430        0.397
KAR4___________1008_residue    0.354        0.383
PBN1___________1251_residue    0.330        0.386
```

```
input.res <- read.table(inputoutfile, header = TRUE)
head(input.res)
```

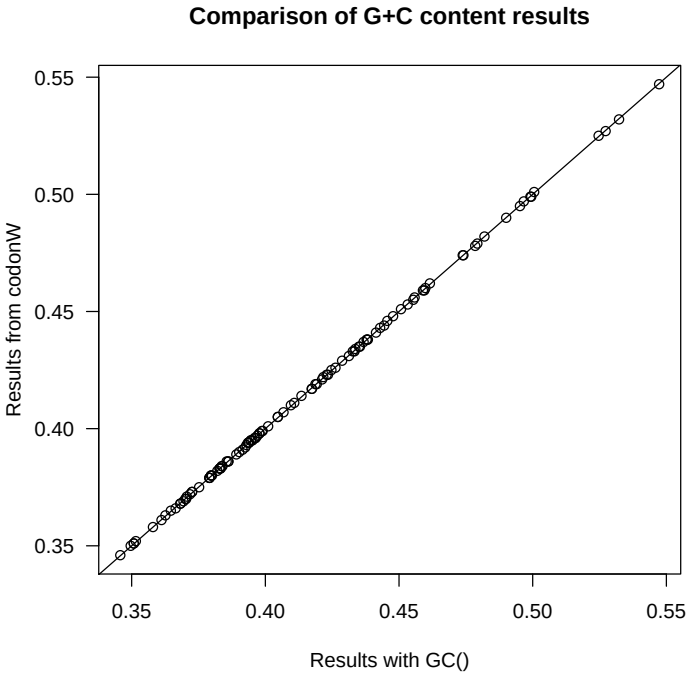
```
                      title  GC3s    GC
1 YCG9_Probable_____________13 0.335 0.394
2 YCG8_________573_residues_ 0.439 0.446
3 ALPHA2_________633_residue 0.328 0.351
4 ALPHA1_________528_residue 0.345 0.379
5 CHA1___________1083_residue 0.328 0.394
6 KRR1___________951_residue 0.364 0.384
```

Let's try to reproduce the results for the G+C content, we know that we have to remove the last stop codon:

```
input.gc <- sapply(input, function(s) GC(s[1:(length(s) -
    3)]))
max(abs(input.gc - input.res$GC))
```

```
[1] 0.0004946237
```

```
plot(x = input.gc, y = input.res$GC, las = 1, xlab = "Results with GC()",
    ylab = "Results from codonW", main = "Comparison of G+C content results")
abline(c(0, 1))
```

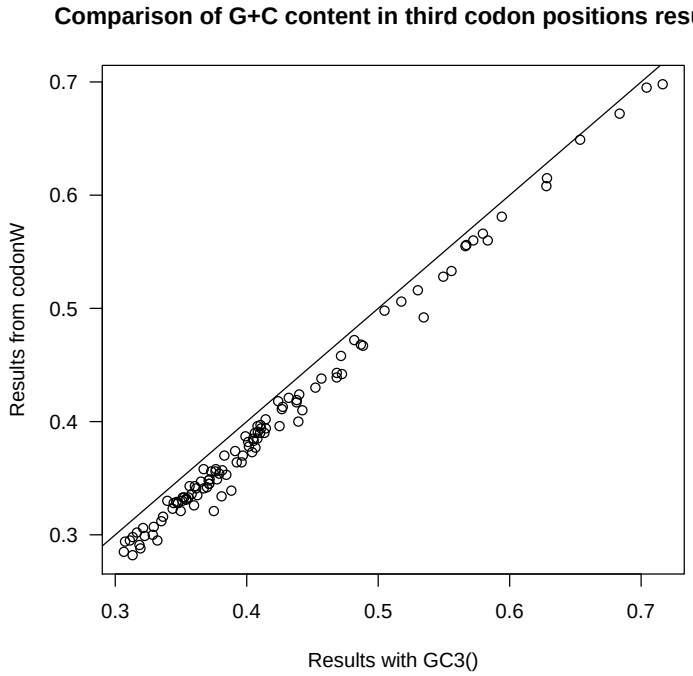


The results are consistent if we consider that we have 3 significant digits in the file `input.out`. Now, let's try to reproduce the results for G+C in third codon positions:

```
input.gc3 <- sapply(input, function(s) GC3(s[1:(length(s) -
    3)]))
max(abs(input.gc3 - input.res$GC3s))
```

```
[1] 0.054
```

```
plot(x = input.gc3, y = input.res$GC3s, las = 1, xlab = "Results with GC3()",
    ylab = "Results from codonW", main = "Comparison of G+C content in third codon positions results")
abline(c(0, 1))
```

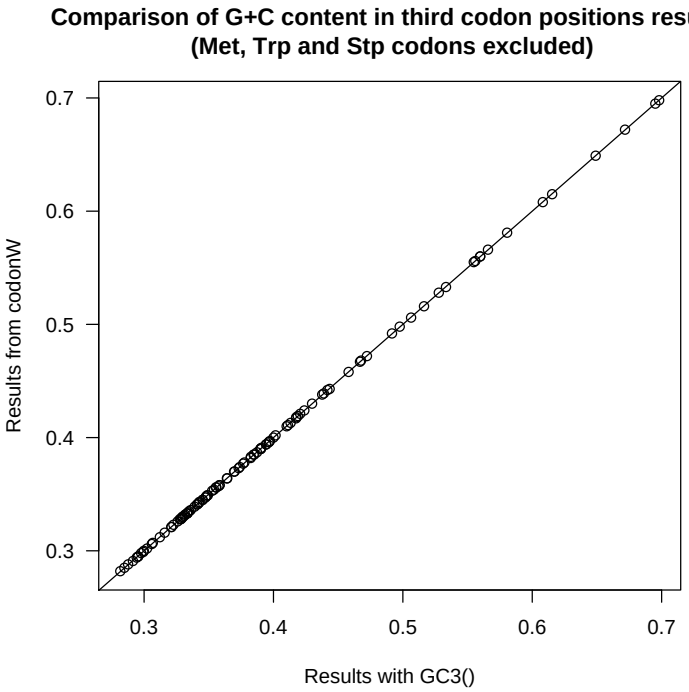


There is clearly a problem here. Looking into the documentation of `codonW`, GC3s is the G+C content in third codon position after removing non-synonymous and stop codons (those corresponding to Met, Trp, Stp). Let's remove these codons:

```
codons <- words()
names(codons) <- sapply(codons, function(c) aaa(translate(s2c(c),
    numcode = 1)))
okcodons <- codons[!names(codons) %in% c("Met", "Trp", "Stp")]
gc3s <- function(s) {
    tmp <- splitseq(s)
    tmp <- tmp[tmp %in% okcodons]
    tmp <- s2c(paste(tmp, collapse = ""))
    GC3(tmp)
}
input.gc3s <- sapply(input, gc3s)
max(abs(input.gc3s - input.res$GC3s))
```

```
[1] 0.0004980843
```

```
plot(x = input.gc3s, y = input.res$GC3s, las = 1, xlab = "Results with GC3()",
    ylab = "Results from codonW", main = "Comparison of G+C content in third codon positions results\n(M
abline(c(0, 1))
```



The results are now consistent. But thinking more about it there is still a problem with the codons for Ile:

```
codons[names(codons) == "Ile"]
```

```
  Ile   Ile   Ile
"ata" "atc" "att"
```

There are three codons for Ile. If the distribution of the four bases was uniform and selectively neutral in third codon position of synonymous codons, then we would expect to get a G+C of 50% in quartet and duet codons at third codons positions because they all have the same number of W (A or T)and S (C or G) bases in third position. But for Ile we have two codons ending in W versus only one in S so that we would get a G+C of $\frac{1}{3}$ instead of $\frac{1}{2}$. This point was clearly stated [54] by Sueoka in 1988:

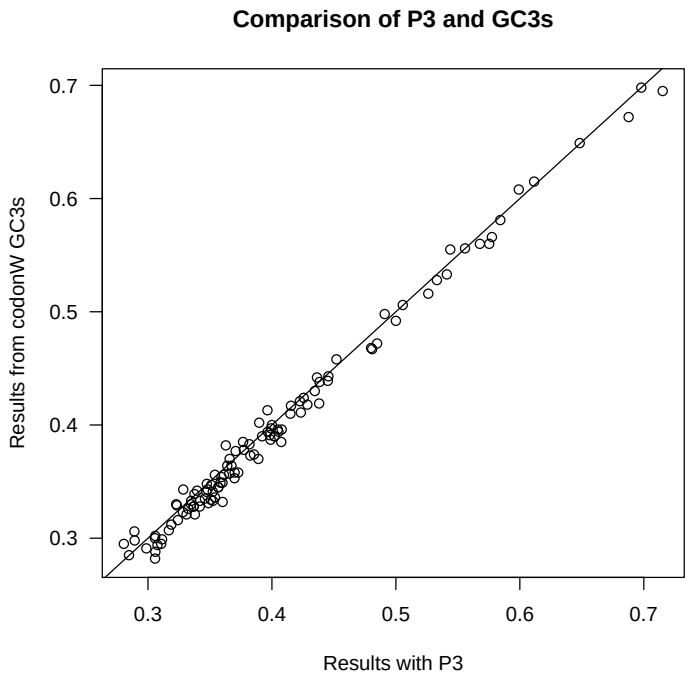
> **G + C Content of the Three Codons Positions.** In the present analysis, observed G + C contents of the first, second, and third codon positions (P_1, P_2, and P_3, respectively) are corrected average G + C contents of the three codon positions that are calculated from 56 triplets out of 64. Because of the inequality of α and γ at the third codon position, the three stop codons (TAA, TAG, and TGA) and the three codons for isoleucine (ATT, ATC, and ATA) were excluded in calculation of P_3, and two single codons for methionine (ATG) and tryptophan (TGG) were excluded in all three (P_1, P_2, and P_3)

Let's compute P_3 and compare it with GC3s:

```
P3codons <- codons[!names(codons) %in% c("Met", "Trp", "Ile",
    "Stp")]
P3 <- function(s) {
    tmp <- splitseq(s)
    tmp <- tmp[tmp %in% P3codons]
    tmp <- s2c(paste(tmp, collapse = ""))
    GC3(tmp)
}
input.P3 <- sapply(input, P3)
max(abs(input.P3 - input.res$GC3s))
```

```
[1] 0.02821505
```

```
plot(x = input.P3, y = input.res$GC3s, las = 1, xlab = "Results with P3",
    ylab = "Results from codonW GC3s", main = "Comparison of P3 and GC3s")
abline(c(0, 1))
```



This is not exactly the same, the maximum observed difference here is about 3%. In practice, P_3, GC3, and GC3s are only slightly different [55].

A.3 How do I get a sequence from its name?

This question is adapted from an e-mail (22 Jun 2006) by Gang Xu. I know that the UniProt (SwissProt) entry of my protein is `P08758`, if I know its name[1], how can I get the sequence?

```
choosebank("swissprot")
query("myprot", "AC=P08758")
getSequence(myprot$req[[1]])
```

[1] More exactly, this is the accession number. Sequence names are not stable over time, it's always better to use the accession numbers.

```
  [1] "A" "Q" "V" "L" "R" "G" "T" "V" "T" "D" "F" "P" "G" "F" "D" "E" "R" "A"
 [19] "D" "A" "E" "T" "L" "R" "K" "A" "M" "K" "G" "L" "G" "T" "D" "E" "E" "S"
 [37] "I" "L" "T" "L" "L" "T" "S" "R" "S" "N" "A" "Q" "R" "Q" "E" "I" "S" "A"
 [55] "A" "F" "K" "T" "L" "F" "G" "R" "D" "L" "L" "D" "D" "L" "K" "S" "E" "L"
 [73] "T" "G" "K" "F" "E" "K" "L" "I" "V" "A" "L" "M" "K" "P" "S" "R" "L" "Y"
 [91] "D" "A" "Y" "E" "L" "K" "H" "A" "L" "K" "G" "A" "G" "T" "N" "E" "K" "V"
[109] "L" "T" "E" "I" "I" "A" "S" "R" "T" "P" "E" "E" "L" "R" "A" "I" "K" "Q"
[127] "V" "Y" "E" "E" "E" "Y" "G" "S" "S" "L" "E" "D" "D" "V" "V" "G" "D" "T"
[145] "S" "G" "Y" "Y" "Q" "R" "M" "L" "V" "V" "L" "L" "Q" "A" "N" "R" "D" "P"
[163] "D" "A" "G" "I" "D" "E" "A" "Q" "V" "E" "Q" "D" "A" "Q" "A" "L" "F" "Q"
[181] "A" "G" "E" "L" "K" "W" "G" "T" "D" "E" "E" "K" "F" "I" "T" "I" "F" "G"
[199] "T" "R" "S" "V" "S" "H" "L" "R" "K" "V" "F" "D" "K" "Y" "M" "T" "I" "S"
[217] "G" "F" "Q" "I" "E" "E" "T" "I" "D" "R" "E" "T" "S" "G" "N" "L" "E" "Q"
[235] "L" "L" "L" "A" "V" "V" "K" "S" "I" "R" "S" "I" "P" "A" "Y" "L" "A" "E"
[253] "T" "L" "Y" "Y" "A" "M" "K" "G" "A" "G" "T" "D" "D" "H" "T" "L" "I" "R"
[271] "V" "M" "V" "S" "R" "S" "E" "I" "D" "L" "F" "N" "I" "R" "K" "E" "F" "R"
[289] "K" "N" "F" "A" "T" "S" "L" "Y" "S" "M" "I" "K" "G" "D" "T" "S" "G" "D"
[307] "Y" "K" "K" "A" "L" "L" "L" "L" "C" "G" "E" "D" "D"
```

A.4 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

APPENDIX B

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Preamble

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APPENDIX C

Genetic codes

Lobry, J.R.

C.1 Standard genetic code

The standard genetic code given in table C.1 was produced with the following R code and inserted with `\input{../tables/stdcode.tex}` within this LaTeX document and referenced as `\ref{stdcode}` in the text.

```
tablecode(latexfile = "../tables/stdcode.tex", label = "stdcode",
    size = "small")
```

C.2 Available genetic code numbers

The genetic code numbers are those from the NCBI[1] (`http://130.14.29.110/Taxonomy/Utils/wprintgc.cgi?mode=c`). This compilation from Andrzej (Anjay) Elzanowski, Jim Ostell, Detlef Leipe, and Vladimir Soussov is based primarily on two previous reviews [41, 23].

```
codes <- SEQINR.UTIL$CODES.NCBI
availablecodes <- which(codes$CODES != "deleted")
codes[availablecodes, "ORGANISMES", drop = FALSE]
```

```
                          ORGANISMES
1                           standard
2           vertebrate.mitochondrial
3                 yeast.mitochondrial
4  protozoan.mitochondrial+mycoplasma
5         invertebrate.mitochondrial
6               ciliate+dasycladacean
9   echinoderm+flatworm.mitochondrial
10                          euplotid
11            bacterial+plantplastid
12                  alternativeyeast
13            ascidian.mitochondrial
14  alternativeflatworm.mitochondrial
15                        blepharism
```

[1] National Center for Biotechnology Information, Bethesda, Maryland, U.S.A.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.1: Genetic code number 1: standard.

```
16        chlorophycean.mitochondrial
21            trematode.mitochondrial
22          scenedesmus.mitochondrial
23       hraustochytrium.mitochondria
```

The tables of variant genetic codes outlining the differences were produced with the following R code:

```
cdorder <- paste(paste(rep(s2c("tcag"), each = 16), s2c("tcag"),
    sep = ""), rep(s2c("tcag"), each = 4), sep = "")
stdcode <- sapply(lapply(cdorder, s2c), translate, numcode = 1)
for (cd in availablecodes[-1]) {
    Tfile <- paste("../tables/codnum", cd, ".tex", sep = "")
    preemph <- "\\textcolor{red}{\\textbf{"
    postemph <- "}}"
    stcodon <- (stdcode == sapply(lapply(cdorder, s2c), translate,
        numcode = cd))
    pre <- ifelse(stcodon, "", preemph)
    post <- ifelse(stcodon, "", postemph)
    tablecode(numcode = cd, latexfile = Tfile, size = "small",
        preaa = pre, postaa = post)
    cat(paste("\\input{", Tfile, "}", sep = ""), sep = "\n")
}
```

C.3 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	**Met**	ACA	Thr	AAA	Lys	**AGA**	**Stp**
ATG	Met	ACG	Thr	AAG	Lys	**AGG**	**Stp**
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.2: Genetic code number 2: vertebrate.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	**Thr**	CCT	Pro	CAT	His	CGT	Arg
CTC	**Thr**	CCC	Pro	CAC	His	CGC	Arg
CTA	**Thr**	CCA	Pro	CAA	Gln	CGA	Arg
CTG	**Thr**	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	**Met**	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.3: Genetic code number 3: yeast.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.4: Genetic code number 4: protozoan.mitochondrial+mycoplasma.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	**Met**	ACA	Thr	AAA	Lys	**AGA**	**Ser**
ATG	Met	ACG	Thr	AAG	Lys	**AGG**	**Ser**
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.5: Genetic code number 5: invertebrate.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	**TAA**	**Gln**	TGA	Stp
TTG	Leu	TCG	Ser	**TAG**	**Gln**	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.6: Genetic code number 6: ciliate+dasycladacean.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	**AAA**	**Asn**	**AGA**	**Ser**
ATG	Met	ACG	Thr	AAG	Lys	**AGG**	**Ser**
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.7: Genetic code number 9: echinoderm+flatworm.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Cys**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.8: Genetic code number 10: euplotid.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.9: Genetic code number 11: bacterial+plantplastid.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	**Ser**	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.10: Genetic code number 12: alternativeyeast.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	**Met**	ACA	Thr	AAA	Lys	**AGA**	**Gly**
ATG	Met	ACG	Thr	AAG	Lys	**AGG**	**Gly**
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.11: Genetic code number 13: ascidian.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	**TAA**	**Tyr**	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	**AAA**	**Asn**	**AGA**	**Ser**
ATG	Met	ACG	Thr	AAG	Lys	**AGG**	**Ser**
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.12: Genetic code number 14: alternativeflatworm.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	**TAG**	**Gln**	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.13: Genetic code number 15: blepharism.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	**TAG**	**Leu**	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.14: Genetic code number 16: chlorophycean.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	TCA	Ser	TAA	Stp	**TGA**	**Trp**
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	**Met**	ACA	Thr	**AAA**	**Asn**	**AGA**	**Ser**
ATG	Met	ACG	Thr	AAG	Lys	**AGG**	**Ser**
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.15: Genetic code number 21: trematode.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	Leu	**TCA**	**Stp**	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	**TAG**	**Leu**	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.16: Genetic code number 22: scenedesmus.mitochondrial.

TTT	Phe	TCT	Ser	TAT	Tyr	TGT	Cys
TTC	Phe	TCC	Ser	TAC	Tyr	TGC	Cys
TTA	**Stp**	TCA	Ser	TAA	Stp	TGA	Stp
TTG	Leu	TCG	Ser	TAG	Stp	TGG	Trp
CTT	Leu	CCT	Pro	CAT	His	CGT	Arg
CTC	Leu	CCC	Pro	CAC	His	CGC	Arg
CTA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CTG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
ATT	Ile	ACT	Thr	AAT	Asn	AGT	Ser
ATC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
ATA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
ATG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GTT	Val	GCT	Ala	GAT	Asp	GGT	Gly
GTC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GTA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GTG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Table C.17: Genetic code number 23: hraustochytrium.mitochondria.

APPENDIX D

Release notes

Lobry, J.R., Palmeira, L.

D.1 release 1.0-6

Release 1.0-6 is a minor release to fix a problem found and solved by Kurt Hornik (namely a change from `SET_ELEMENT` to `SET_STRING_ELT` in C code for `s2c()` in file `util.c`). The few changes are as follows.

- More typographical option for the output LaTeX table of `tablecode()` are now available to outline deviations from the standard genetic code (see example in the appendix "genetic codes" of the manual).
- A new dataset `aaindex` extracted from the aaindex database [26, 56, 40] is now available. It contains a list of 544 physicochemical and biological properties for the 20 amino-acids
- The default value for argument `dia` is now `FALSE` in function `tablecode()`.
- The example code for `data(chargaff)` has been changed.

D.2 release 1.0-5

- A new function `dotPlot()` is now available.
- A new function `crelistfromclientdata()` is now available to create a list on the server from a local file of sequence names, sequence accession numbers, species names, or keywords names.
- A new function `pmw()` to compute the molecular weight of a protein is now available.

- A new function `reverse.align()` contributed by Anamaria Necşulea is now available to align CDS at the protein level and then reverse translate this at the nucleic acid level from a `clustalw` output. This can be done on the fly if `clustalw` is available on your platform.

- An undocumented behavior was reported by Guy Perrière for `uco()` when computing RSCU on sequences where an amino-acid is missing. There is now a new argument `NA.rscu` that allows the user to force the missing values to his favorite magic value.

- There was a bug in `read.fasta()`: some sequence names were truncated, this is now fixed (thanks to Marcus G. Daniels for pointing this). In order to be more consistent with standard functions such as `read.table()` or `scan()`, the file argument starts now with a lower case letter (`file`) in function `read.fasta()`, but the old-style `File` is still functional for forward-compatibility. There is a new logical argument in `read.fasta()` named `as.string` to allow sequences to be returned as strings instead of vector of single characters. The automatic conversion of DNA sequences into lower case letters can now be disabled with the new logical argument `forceDNAtolower`. It is also possible to disable the automatic attributes settings with the new logical argument `set.attributes`.

- A new function `write.fasta()` is now available.

- The function `kaks()` now forces character in sequences to upper case. This default behavior can be neutralized in order to save time by setting the argument `forceUpperCase` to `FALSE`.

D.3 release 1.0-4

- The scaling factor $n_{\bullet\bullet}$ was missing in equation 5.3.

- The files `louse.fasta`, `louse.names`, `gopher.fasta`, `gopher.names` and `ortho.fasta` that were used for examples in the previous version of this document are no more downloaded from the internet since they are now distributed in the `sequences/` folder of the package.

- An example of synonymous and non synonymous codon usage analysis was added to the vignette along with two toy data sets (`toyaa` and `toycodon`).

- A FAQ section was added to the vignette.

- A bug in `getAnnot()` when the number of lines was zero is now fixed.

- There is now a new argument, `latexfile`, in `tablecode()` to export genetic codes tables in a LaTeX document, for instance table 1.2 and table 1.3 here.

- There is now a new argument, `freq`, in `count()` to compute word frequencies instead of counts.

- Function `splitseq()` has been entirely rewritten to improve speed.

- Functions computing the G+C content: `GC()`, `GC1()`, `GC2()`, `GC3()` were rewritten to improve speed, and their document files were merged to facilitate usage.
- The following new functions have been added:
  - `syncodons()` returns all synonymous codons for a given codon. Argument `numcode` specifies the desired genetic code.
  - `ucoweight()` returns codon usage bias on a sequence as the number of synonymous codons present in the sequence for each amino acid.
  - `synsequence()` generates a random coding sequence which is synonymous to a given sequence and has a chosen codon usage bias.
  - `permutation()` generates a new sequence from a given sequence, while maintaining some constraints from the given sequence such as nucleotide frequency, codon usage bias, ...
  - `rho()` computes the rho statistic on dinucleotides as defined in [25].
  - `zscore()` computes the zscore statistic on dinucleotides as defined in [42].
- Two datasets (`dinucl` and `prochlo`) were added to illustrate these new functions.

D.4 release 1.0-3

- The new package maintainer is Dr. Simon Penel, PhD, who has now a fixed position in the laboratory that issued **seqinR** (`penel@biomserv.univ-lyon1.fr`). Delphine Charif was successful too to get a fixed position in the same lab, with now a different research task (but who knows?). Thanks to the close vicinity of our pioneering maintainers the transition was sweet. The DESCRIPTION file of the **seqinR** package has been updated to take this into account.
- The reference paper for the package is now *in press*. We do not have the full reference for now, you may use `citation("seqinr")` to check if it is complete now:

```
citation("seqinr")

To cite seqinR in publications use:

  in the body of the text (J.R. Lobry, personal communication), or
  wait for the exact complete reference.

A BibTeX entry for LaTeX users is

  @incollection{,
    author = {D. Charif and J.R. Lobry},
    title = {SeqinR 1.0-2: a contributed package to the R project for statistical computing devoted to biologic
    booktitle = {Structural approaches to sequence evolution: Molecules, networks, populations},
    year = {2006},
    editor = {U. Bastolla, M. Porto, H.E. Roman and M. Vendruscolo},
    volume = {NA},
    series = {Biological and Medical Physics, Biomedical Engineering},
    pages = {NA},
    address = {New York},
    month = {NA},
    organization = {NA},
```

```
    publisher = {Springer Verlag},
    note = {in press},
  }

Note that the orginal article and updates are available in the
../../../../../seqinr.Rcheck/seqinr/doc/ folder in PDF format
```

- There was a bug when sending a `gfrag` request to the server for long (Mb range) sequences. The length argument was converted to scientific notations that are not understand by the server. This is now corrected and should work up the the Gb scale.
- The `query()` function has been improved by de-looping list element info request, there are now download at once which is much more efficient. For example, a query from a researcher-home ADSL connection with a list with about 1000 elements was 60 seconds and is now only 4 seconds (*i.e.* 15 times faster now).
- A new parameter `virtual` has been added to `query()` so that long lists can stay on the server without trying to download them automatically. A query like `query(s$socket,"allcds","t=cds", virtual = TRUE)` is now possible.
- Relevant genetic codes and frames are now automatically propagated.
- `SeqinR` sends now its name and version number to the server.
- Strict control on ambiguous DNA base alphabet has been relaxed.
- Default value for parameter `invisible` of function `query()` is now `TRUE`.

D.5 Session Informations

This part was compiled under the following R environment:

- Version 2.3.1 (2006-06-01), `powerpc-apple-darwin8.6.0`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: MASS 7.2-27.1, ade4 1.4-1, ape 1.8-2, gee 4.13-10, lattice 0.13-8, nlme 3.1-73, seqinr 1.0-6, xtable 1.3-0

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