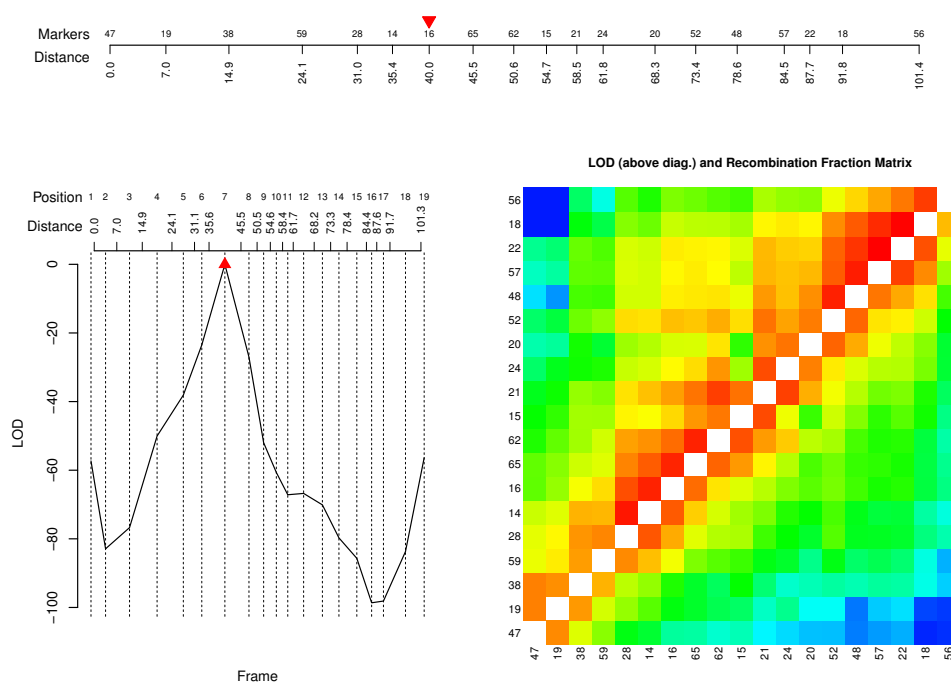


OneMap Tutorial

Software for constructing genetic maps in experimental crosses: full-sib, RILs, F_2 and back-crosses

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1 Overview

OneMap is an environment for constructing linkage maps in several experimental crosses, including outcrosses (full-sib families derived from two non-homozygous parents), RILs, F_2 and backcrosses. It is implemented as a package to be used under the freely distributed R software, which is a language and environment for statistical computing (www.r-project.org). It is designed to be fully integrated with R/qtl package (Broman et al., 2008) and Windows QTL Cartographer (Wang et al., 2010) in order to do QTL mapping.

Wu et al. (2002a) proposed a methodology to construct genetic maps in outcrossing species, which allows the analysis of a mixed set of different marker types containing various segregation patterns. It also allows the simultaneous estimation of linkage and linkage phases between markers, and was successfully applied in the analysis of sugarcane (Garcia et al., 2006; Oliveira et al., 2007) and *Passiflora* (Oliveira et al., 2008) data sets. Actually, the analysis of these data sets motivated the implementation of the first release of *OneMap* (Margarido et al., 2007).

After extensively testing the software, we noticed that the construction of linkage maps could be greatly enhanced with the use of multipoint likelihood through Hidden Markov Models (HMM). Jiang and Zeng (1997) explained in detail this methodology, emphasizing its advantages and limitations for populations derived from inbred lines. Merging the ideas of Wu et al. (2002a) and the HMM framework, as done by Wu et al. (2002b), we then developed version 1.0-0 of *OneMap*, which could order markers using HMM-based algorithms for outcrossing species, in a similar way as implemented in MAPMAKER/EXP (Lander et al., 1987). We verified the great advantages of the new procedure through extensive simulations.

In version 2.0-0, we included several major modifications to take advantage of the fact that some segregation patterns that occur in outcrossing populations can also occur in populations derived from inbred lines (*i.e.*, RILs, F_2 and backcrosses). For example, a marker that segregates in a 1 : 2 : 1 fashion in an outcrossing context can be viewed as a co-dominant marker in F_2 populations. The main difference is that, for the latter, there is no need to estimate linkage phases. Using these ideas, we adapted *OneMap* to also construct genetic maps in RILs, F_2 and backcross populations, taking advantage of *OneMap* capabilities. Moreover, we also implemented three new ordering algorithms besides the ones included in version 1.0-0: Rapid Chain Delineation - RCD (Doerge, 1996) and TRY (Lander et al., 1987). They are Seriation - SER (Buetow and Chakravarti, 1987), recombination counting and ordering - RECORD (Van Os et al., 2005) and unidirectional growth - UG (Tan and Fu, 2006). They can be used for all experimental crosses included in *OneMap*, and can be chosen to give the best result for any

situation faced by the user (Mollinari et al., 2009).

OneMap is available as source code for WindowsTM and Unix systems. It is released under the GNU General Public License, is open-source and the code can be changed freely. It comes with no warranty.

Although no advanced knowledge in R is required to use *OneMap*, in Section 2 we present a short introduction to R software, where we address the basic knowledge required to start using *OneMap*. People with some knowledge of R may just skip this part. In Section 3, information about *OneMap* installation is provided. In Section 4, we show the usage of *OneMap* functions for outcrossing (non-inbred) populations. In Section 5 we do the same for F₂ populations, which can also be applied to backcrosses and RILs. All sections may be read independently.

1.1 Citation

Margarido, G.R.A., Souza, A.P. and Garcia, A.A.F. OneMap: software for genetic mapping in outcrossing species. *Hereditas* 144: 78-79, 2007.

2 Introduction to R

R is a language and environment for statistical computing and graphics. To download R, please visit the Comprehensive R Archive Network (cran.r-project.org). Although we prefer and recommend the Linux version, in this tutorial, it is assumed that the user is running WindowsTM. Users of R under Linux or Mac[®] OS should have no difficulty following this tutorial.

After installing R, you can launch it by double-clicking the R icon created on your desktop during the installation process. You will see a window with the R Console (Figure 1).

2.1 Getting started

In Figure 1, you can see a *greater than* sign (“*>*”), which means that R is waiting for a command. We call this *prompt*. Let us start with a simple example adding two numbers. Type “2 + 3” at the prompt then type the **Enter** key:

```
2 + 3
## [1] 5
```

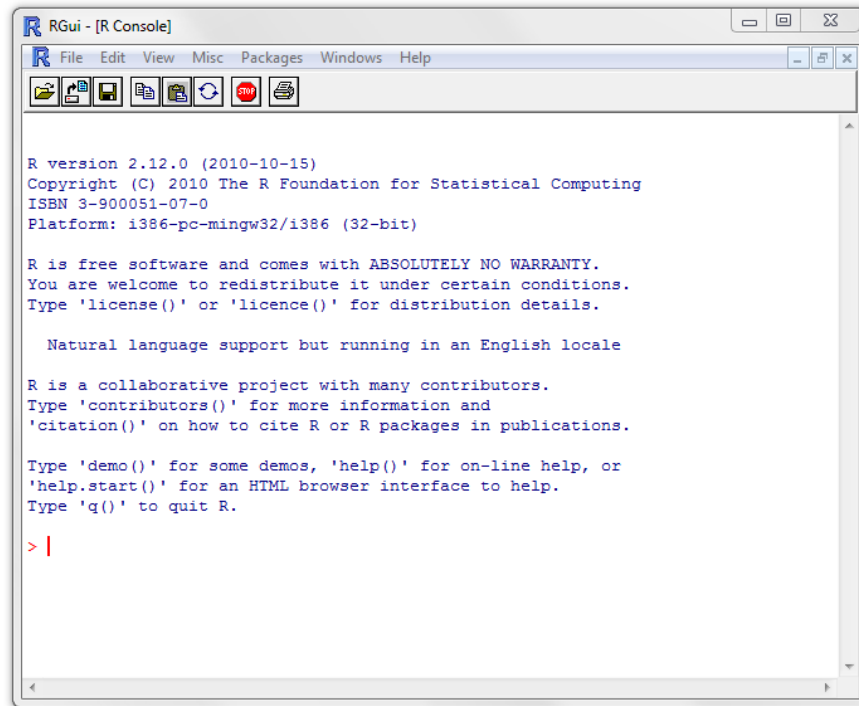


Figure 1: The R Console.

You can see the result directly on the screen. You can store this result into a variable for future use, applying the assignment operator `<-` (*less than sign and minus*, altogether):

```
x <- 2 + 3
```

The result of the calculation was stored into the variable `x`. You can access this result typing “`x`” at the prompt:

```
x  
## [1] 5
```

You can also use the variable `x` into another calculation, for example:

```
x + 4  
## [1] 9
```

2.2 Functions

Another fundamental aspect in R is the usage of *functions*. A function is a predefined routine used to do specific calculations. For example, to calculate the natural logarithm of 6.7, we can use the function `log`:

```
log(6.7)

## [1] 1.902108
```

The function `log` contains a group of internal procedures to calculate the natural logarithm of a positive real number. The input values of a function are called *arguments*. In the previous example, we provided only one argument to the function (6.7). Sometimes a function has more than one argument. For example, to obtain the logarithm of 6.7 to base 4, you can use:

```
log(6.7, base = 4)

## [1] 1.372081
```

It is possible to calculate the natural logarithm of a set of numbers by defining a vector and using it as the first argument of the function `log`. To do so we use the function `c`, that *combines* a set of values into a vector. Thus, to calculate the logarithm of the numbers 6.7, 3.2, 5.4, 8.1, 4.9, 9.7 and 2.5, we can use:

```
y <- c(6.7, 3.2, 5.4, 8.1, 4.9, 9.7, 2.5)
log(y)

## [1] 1.9021075 1.1631508 1.6863990
## [4] 2.0918641 1.5892352 2.2721259
## [7] 0.9162907
```

2.3 Getting help

Every R function has a help page which can be accessed using a question mark before the name of the function. For example, to get help on function `log`, you would type:

```
?log
```

This command will open a help page in the default web browser of your system. The help page contains some important information about the function such its syntax, its arguments and some usage examples.

2.4 Packages

Although R has a huge amount of internal functions, for doing more specific computations, like constructing genetic linkage maps, it is necessary to use complementary functions. These functions can be obtained by installing a *package*. A package is a collection of related functions, help files and example data files that have been bundled together (Adler, 2010).

For example, let us assume you need to convert a set of recombination fractions into centimorgan distance using the Kosambi function. One possible way to do that is to use the basic R functions to calculate the distances. Another way is use the *OneMap* package. To install *OneMap* you can type:

```
setRepositories(ind = 1:2)
install.packages("onemap")
```

You also can use the console menus: *Packages* → *Install package(s)*. After clicking, a box will pop-up asking you to choose the CRAN mirror. Choose the location nearest to you. Then, another box will pop-up asking you to choose the package you want to install. Select **onemap** then click *OK*. The package will be automatically installed on your computer. Returning to the console, you need to load *OneMap* by typing:

```
library(onemap)

## Loading required package: tcltk
## Loading required package: tkrplot
## Loading required package: ggplot2
## Want to understand how all the
## pieces fit together? Buy the
## ggplot2 book:
## http://ggplot2.org/book/
```


Let us enter some recombination fractions, for example, 0.01, 0.12, 0.05, 0.11, 0.21, 0.07, and save it into a variable called `rf`:

```
rf <- c(0.01, 0.12, 0.05, 0.11, 0.21, 0.07)
```

Now, let us use the function `kosambi`, which belongs to *OneMap* package, to do the calculation:

```
kosambi(rf)

## [1] 1.000133 12.238706 5.016767
## [4] 11.182805 22.384601 7.046279
```

You can also obtain help on the function `kosambi` using the question mark in the same way as done with function `log`:

```
?kosambi
```

2.5 Importing and exporting data

So far, we entered the variables in R by typing them directly into the console. However, in real situations we usually *read* these values from a file or a data bank. To exemplify this procedure, copy and paste the following table into a text editor (for example, *notepad*) and save it to a file called `test.txt` into your *working directory* (such as *My Documents*).

x	y
2.13	4.50
4.48	1.98
10.95	9.29
10.03	16.25
12.72	27.38
24.63	22.60
22.57	36.87
29.78	31.73
19.54	10.42
7.86	14.68
11.75	8.68
23.71	37.39

To read these data in R, we have to first set the working directory using the function `setwd`. For example, if `"C:/Users/mmollina/Documents"` is the full path to *My Documents* directory, one should use:

```
setwd("C:/Users/mmollina/Documents")
```

Every time you inform paths, directories or files you have to use double quotes (“ ”), which indicate a string of characters instead of a variable name. You can also use the console menus to set the working directory: *File* → *Change Dir...* From here, every object will be read or saved in this directory.

Now let us read the file `test.txt` into R and store it in a variable called `dat` using the function `read.table`. The first argument is the name of the file. The second indicates if the file contains a header, that is, if the first line of the file contains the names of the variables:

```
(dat <- read.table(file = "test.txt", header = TRUE))
```

```
##      x      y
## 1  2.13  4.50
## 2  4.48  1.98
## 3 10.95  9.29
## 4 10.03 16.25
## 5 12.72 27.38
## 6 24.63 22.60
## 7 22.57 36.87
## 8 29.78 31.73
## 9 19.54 10.42
## 10  7.86 14.68
## 11 11.75  8.68
## 12 23.71 37.39
```

Notice that the whole command line is enclosed within parentheses. This indicates R to show the results at the same time you store them into a variable. One could type the command without parenthesis and then type `dat` at the prompt, to produce the same result. Inspecting the object `dat` you can see a table with 12 rows and two columns. The names of the columns are `x` and `y`. We can access the variables in columns using the dollar sign followed by the column name:

```

dat$x

## [1]  2.13  4.48 10.95 10.03 12.72 24.63
## [7] 22.57 29.78 19.54  7.86 11.75 23.71

dat$y

## [1]  4.50  1.98  9.29 16.25 27.38 22.60
## [7] 36.87 31.73 10.42 14.68  8.68 37.39

```

It is also possible to use a function called **summary** to extract some information about the object **dat** or about each one of the columns separately::

```

summary(dat)

##           x           y
## Min.      : 2.130   Min.      : 1.980
## 1st Qu.: 9.488   1st Qu.: 9.137
## Median :12.235   Median :15.465
## Mean     :15.012   Mean      :18.481
## 3rd Qu.:22.855   3rd Qu.:28.468
## Max.     :29.780   Max.       :37.390

summary(dat$x)

##      Min. 1st Qu.  Median      Mean 3rd Qu.
##    2.130  9.487  12.240  15.010  22.860
##      Max.
##    29.780

summary(dat$y)

##      Min. 1st Qu.  Median      Mean 3rd Qu.
##    1.980  9.138  15.460  18.480  28.470
##      Max.
##    37.390

```

The function `summary` provides some basic statistics about the variables in the dataset. If you want to export this information to a file you can use the function `write.table`:

```
write.table(x = summary(dat), file = "test_sum.txt", quote = FALSE)
```

The first argument is the output of the `summary` function. Note that it is possible to use a function as an argument of another one. The second argument is the name of the file in which the summary is going to be written. Notice that the file will be written in the *working directory*, previously set. The third argument eliminates double quotes from the output file. After running the command, you can look for the file `test_sum.txt` in the *working directory*.

2.6 Classes and methods

In R, every object belongs to a *class*. For example, the object `dat` belongs to a class called `data.frame`. We can obtain this information using the function `class`:

```
class(dat)

## [1] "data.frame"
```

When we use the function `summary`, it recognizes the class of the `dat` object and applies a specific procedure to the `data.frame` class, which in this case involves the computation of some descriptive statistics. This procedure is called *method*. However, other classes of objects can be used as arguments to function `summary` and the result will be different. For example, let us adjust a linear model using column `y` as the dependent variable and column `x` as independent. This can be done with the function `lm()`:

```
ft_mod <- lm(dat$y ~ dat$x)
ft_mod

##
## Call:
## lm(formula = dat$y ~ dat$x)
##
## Coefficients:
## (Intercept)      dat$x
##      1.803      1.111
```

Function `lm` is used to fit linear models and, by default, prints just a formula and the coefficients of the linear regression. Object `ft_mod` is of class `lm`:

```
class(ft_mod)

## [1] "lm"
```

To obtain more information about the fitted model, we can use the function `summary`:

```
summary(ft_mod)

##
## Call:
## lm(formula = dat$y ~ dat$x)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -13.091  -5.144  -1.413   5.421  11.446
##
## Coefficients:
##              Estimate Std. Error t value
## (Intercept)   1.8026     4.7689   0.378
## dat$x         1.1110     0.2771   4.009
##              Pr(>|t|)
## (Intercept)  0.71334
## dat$x        0.00248
##
## Residual standard error: 8.075 on 10 degrees of freedom
## Multiple R-squared:  0.6164, Adjusted R-squared:  0.5781
## F-statistic: 16.07 on 1 and 10 DF,  p-value: 0.002482
```

In this case, the function `summary` recognizes `ft_mod` as an object of class `lm` and applies a method which shows information about the fitted model such as the distribution of residuals, regression coefficients, t -tests, the coefficient of determination (R^2), etc (significance stars not shown). Thus, it is possible to use the same function on different classes of objects to obtain different results. This concept is very important in *OneMap*. For example, depending on the

class of the dataset, which can be `outcross`, `f2`, `intercross`, `backcross`, `riself` and `risib`, a certain set of procedures will be applied.

2.7 Saving a Workspace

You can save your analysis using the function `save.image`. For example, if you want to save your analysis in a file called `myworkspace.RData`, you should use:

```
save.image("myworkspace.RData")
```

You can also use the console menus: *File* → *Save Workspace*. Now, you can load your analysis into R, using the function `load`:

```
load("myworkspace.RData")
```

This is useful if you want to stop one session and continue on the following day, etc.

3 Installation and Introduction to OneMap

OneMap can be installed by opening R and typing the command

```
setRepositories(ind = 1:2)  
install.packages("onemap")
```

You also can use the console menus: *Packages* → *Install package(s)*. After clicking, a box will pop-up asking you to choose the CRAN mirror. Choose the location nearest to you. Then, another box will pop-up asking you to choose the package you want to install. Select `onemap` then click *OK*. The package will be automatically installed on your computer.

OneMap can also be installed by downloading the appropriate files directly from the CRAN web site and following the instructions given in the section “6.3 Installing Packages” of the “R Installation and Administration” manual (<http://cran.r-project.org/doc/manuals/R-admin.pdf>).

OneMap is comprised of a set of functions (listed on Table 1). There are other functions used internally by the software. However, you do not need to use them directly.

After *OneMap* is installed, you can load it with

Table 1: *OneMap* functions

Function type	Function name	Function description
Input	read_onemap	Read data from a OneMap object
	read_mapmaker	Read data from a Mapmaker raw file
Data manipulation	make_seq	Creates a sequence of markers based on objects of other types
	marker_type	Informs the segregation type of genetic markers
	add_marker	Adds markers to a sequence
	drop_marker	Drops markers from a sequence
Genetic mapping	rf.2pts	Estimates recombination fractions (two points)
	group	Assigns markers to linkage groups
	set_map_fun	Defines the default mapping function
	rcd	Orders markers in a sequence using RCD algorithm
	seriation	Orders markers in a sequence using SERIATION algorithm
	record	Orders markers in a sequence using RECORD algorithm
	ug	Orders markers in a sequence using UG algorithm
	compare	Compares all possible orders of markers in a sequence
	try_seq	Tries to map a marker into a given linkage group
	order_seq	Automates map construction through “compare” and “try_seq” functions
	ripple_seq	Compares alternative orders for a map and displays the plausible ones
	map	Constructs a multipoint linkage map for a sequence in a given order
	rf_graph_table	Plots a pairwise recombination fraction and LOD matrix using a color scale.
	draw_map	Draws a genetic map
Output	write_map	Writes a genetic map to a file to be used in other software (only for backcrosses, F_2 and RILs)

```
library(onemap)
```

A list of packages and datasets that are available on your computer can be obtained with

```
library()  
data()
```

4 Outcrossing populations

The following example is intended to show the usage of *OneMap* functions for linkage mapping in **outcrossing** (non-inbred) populations. With basic knowledge of R syntax, one should have no big problems using it. If you are not familiar with R software, we recommend reading Section 2. It is assumed that the user is running WindowsTM. Hopefully these examples will be clear enough to help any user to understand its functionality and start using it.

1. Start R by double-clicking its icon.
2. Load *OneMap*, after installing it:

```
library(onemap)
```

3. To save your project anytime, type:

```
save.image("C:/.../yourfile.RData")
```

or access the toolbar File → Save Workspace.

4.1 Creating the data file

This step may be quite difficult, because the data file is not very simple and some errors can occur while reading it. The input file format is similar to that used by MAPMAKER/EXP (Lander et al., 1987), so experienced users of genetic analysis software should be already familiar with it.

Basically, the input file is a text file, where the first line indicates the cross type and the second line provides information about the number of individuals, the number of markers, the presence of physical marker locations and the presence of phenotypic data. The third line contains sample IDs. Then, the genotype information is included separately for each marker. The character “*” indicates the beginning of information input for a new marker, followed by

the marker name. Next, there is a code indicating the marker type, according to Wu's et al. (2002a) notation (Table 2)

Table 2: Notation used to identify markers and genotypes

crosstype	Parent			Offspring	
	Cross	Observed bands		Observed bands	Segregation
A	1	<i>ab</i> × <i>cd</i>	<i>ab</i> × <i>cd</i>	<i>ac, ad, bc, bd</i>	1:1:1:1
	2	<i>ab</i> × <i>ac</i>	<i>ab</i> × <i>ac</i>	<i>a, ac, ba, bc</i>	1:1:1:1
	3	<i>ab</i> × <i>co</i>	<i>ab</i> × <i>c</i>	<i>ac, a, bc, b</i>	1:1:1:1
	4	<i>ao</i> × <i>bo</i>	<i>a</i> × <i>b</i>	<i>ab, a, b, o</i>	1:1:1:1
B B ₁	5	<i>ab</i> × <i>ao</i>	<i>ab</i> × <i>a</i>	<i>ab, 2a, b</i>	1:2:1
	6	<i>ao</i> × <i>ab</i>	<i>a</i> × <i>ab</i>	<i>ab, 2a, b</i>	1:2:1
	7	<i>ab</i> × <i>ab</i>	<i>ab</i> × <i>ab</i>	<i>a, 2ab, b</i>	1:2:1
C	8	<i>ao</i> × <i>ao</i>	<i>a</i> × <i>a</i>	<i>3a, o</i>	3:1
D D ₁	9	<i>ab</i> × <i>cc</i>	<i>ab</i> × <i>c</i>	<i>ac, bc</i>	1:1
	10	<i>ab</i> × <i>aa</i>	<i>ab</i> × <i>a</i>	<i>a, ab</i>	1:1
	11	<i>ab</i> × <i>oo</i>	<i>ab</i> × <i>o</i>	<i>a, b</i>	1:1
	12	<i>bo</i> × <i>aa</i>	<i>b</i> × <i>a</i>	<i>ab, a</i>	1:1
	13	<i>ao</i> × <i>oo</i>	<i>a</i> × <i>o</i>	<i>a, o</i>	1:1
	14	<i>cc</i> × <i>ab</i>	<i>c</i> × <i>ab</i>	<i>ac, bc</i>	1:1
	15	<i>aa</i> × <i>ab</i>	<i>a</i> × <i>ab</i>	<i>a, ab</i>	1:1
	16	<i>oo</i> × <i>ab</i>	<i>o</i> × <i>ab</i>	<i>a, b</i>	1:1
	17	<i>aa</i> × <i>bo</i>	<i>a</i> × <i>b</i>	<i>ab, a</i>	1:1
	18	<i>oo</i> × <i>ao</i>	<i>o</i> × <i>a</i>	<i>a, o</i>	1:1

Actually, it is recommended to check Wu's et al. (2002a) paper before using *OneMap*. Marker types for outcrosses must be one of the following: *A.1*, *A.2*, *A.3*, *A.4*, *B1.5*, *B2.6*, *B3.7*, *C.8*, *D1.9*, *D1.10*, *D1.11*, *D1.12*, *D1.13*, *D2.14*, *D2.15*, *D2.16*, *D2.17* or *D2.18*, each one corresponding to a row of the table. The letter and the number before the dot indicate the segregation type (*i.e.*, 1:1:1:1, 1:2:1, 3:1 or 1:1), while the number after the dot indicates the observed bands in the offspring. The paper cited above gives details with respect to marker types; we will not discuss them here, but it is easy to see that each marker is classified based on the band patterns of parents and progeny.

Finally, after each marker name, comes the genotype data for the segregating population. The coding for marker genotypes used by *OneMap* is also the same one proposed by Wu et al. (2002a) and the possible values vary according to the specific marker type. Missing data are indicated with the character “-” (minus sign) and an empty space separates the information for each individual. Phenotype information, if present, follows genotypic data with a similar

structure. Details are found in the help of function `read_onemap`.

Here is an example of such file for 10 individuals and 5 markers (the three zeros in the second line indicate that there is no chromosome information, physical position information or phenotypic data, respectively):

```
data type outcross
10 5 0 0 0
I1 I2 I3 I4 I5 I6 I7 I8 I9 I10
*M1 B3.7 ab ab - ab b ab ab - ab b
*M2 D2.18 o - a a - o a - o o
*M3 D1.13 o a a o o - a o a o
*M4 A.4 ab b - ab a b ab b - a
*M5 D2.18 a a o - o o a o o o
```

Notice that once the marker type is identified, no variations of symbols presented on the table for the “observed bands” is allowed. For example, for *A.1*, only *ac*, *ad*, *bc* and *bd* genotypes are expected (plus missing values). We notice that this is a common mistake made by users, so be careful.

The input file must be saved in text format, with extensions like “.raw”. It is a good idea to open the text file called “example_out.raw” (available with *OneMap* and saved in the directory you installed it) to see how this file should be. You can see where *OneMap* is installed using the command

```
system.file(package = "onemap")
```

4.2 Importing data

1. Once the input file is created, data can be loaded and saved into an R object. The function used to import data is named `read_onemap`. Its usage is quite simple:

```
example_out <- read_onemap("C:/workingdirectory", "example_out.raw")
```

```
## Working...
##
## --Read the following data:
## Type of cross:          outcross
## Number of individuals:  100
## Number of markers:     30
## Chromosome information: no
## Position information:   no
## Number of traits:      3
## Missing trait values:
##   Pheno1: 0
##   Pheno2: 3
##   Pheno3: 0
```

The first argument is the directory where the input file is located, so modify it accordingly. The second one is the data file name. In this example, an object named `example_out` was created. If you leave the argument `dir` blank, the file will be read from your *working directory*.

2. You can change the working directory in R using function `setwd()` or in the toolbar clicking File → Change dir (see Section 2.5). If you set your working directory to the one containing the input file, you can just type:

```
example_out <- read_onemap(file = "example_out.raw")
```

If no error has occurred, a message will display some basic information about the data, such as number of individuals and number of markers:

```
## Working...
##
## --Read the following data:
## Type of cross:          outcross
## Number of individuals:  100
## Number of markers:     30
## Chromosome information: no
```

```
## Position information:    no
## Number of traits:      3
## Missing trait values:
##   Pheno1: 0
##   Pheno2: 3
##   Pheno3: 0
```

3. Because this particular data set is distributed along with the package, as an alternative you can load it typing

```
data(example_out)
```

4. Loading the data creates an object of class `onemap`, which will further be used in the analysis. R command `print` recognizes objects of this class. Thus, if you type

```
example_out
```

you will see some information about the object.

```
## This is an object of class 'onemap'
##   Type of cross:      outcross
##   No. individuals:    100
##   No. markers:       30
##   CHROM information:  no
##   POS information:    no
##   Percent genotyped: 100
##
##   Segregation types:
##           A.1 --> 3
##           A.2 --> 1
##           A.4 --> 4
##           B1.5 --> 1
##           B2.6 --> 2
##           B3.7 --> 5
```

```
##           C.8 --> 2
##           D1.10 --> 2
##           D1.12 --> 1
##           D1.13 --> 2
##           D2.15 --> 1
##           D2.16 --> 2
##           D2.17 --> 2
##           D2.18 --> 2
##
##      No. traits:           3
##      Missing trait values:
##      Pheno1: 0
##      Pheno2: 3
##      Pheno3: 0
```

4.3 Estimating two-point recombination fractions

1. To start the analysis, the first step is estimating the recombination fraction between all pairs of markers, using two-point tests:

```
twopts <- rf_2pts(example_out)
```

The function `rf_2pts` uses as default values of LOD Score 3 and maximum recombination fraction 0.50.

2. Different values for the criteria can be chosen using:

```
twopts <- rf_2pts(example_out, LOD = 3, max.rf = 0.4)
```

3. Although two-point tests were implemented in C language, which is usually much faster than R, this step can take quite some time, depending on the number of markers involved and their segregation type, because all combinations will be estimated and tested. Besides, the results use a lot of memory and a rather powerful computer is needed.

4. When the two-point analysis is finished, an object of class `rf_2pts` is created. Typing

```
twopts
```

will show a message with the criteria used in the analysis and some other information:

```
## This is an object of class 'rf_2pts'
##
## Criteria: LOD = 3 , Maximum recombination fraction = 0.4
##
## This object is too complex to print
## Type 'print(object, c(mrk1=marker, mrk2=marker))' to see
## the analysis for two markers
## mrk1 and mrk2 can be the names or numbers of both markers
```

5. If you want to see the results for given markers, say **M1** and **M3**, the command is:

```
print(twopts, c("M1", "M3"))

## Results of the 2-point analysis for markers: M1 and M3
## Criteria: LOD = 3 , Maximum recombination fraction = 0.4
##
##          rf          LOD
## CC 0.2954514 1.646878
## CR 0.2954514 1.646878
## RC 0.7045486 1.646878
## RR 0.7045486 1.646878
```

Each line corresponds to a possible linkage phase. **CC** denotes coupling phase in both parents, **CR** and **RC** denote coupling phase in parent 1 and 2, respectively, and repulsion in the other, and **RR** denotes repulsion phase in both parents. Value **rf** is the maximum likelihood estimate of the recombination fraction, with its corresponding LOD Score.

4.4 Assigning markers to linkage groups

1. Once the recombination fractions and linkage phases for all pairs of markers have been estimated and tested, markers can be assigned to linkage groups. To do this, first use the function `make_seq` to create a sequence with the markers you want to assign:

```
mark_all <- make_seq(twopts, "all")
```

The function `make_seq` is used to create sequences from objects of several kinds, as will be seen along this tutorial. Here, the object is of class `rf_2pts` and the second argument specifies which markers one wants to use. In this example, the argument `"all"` indicates that all markers will be analyzed. If one wants to use only a subset of markers, say `M1` and `M2`, the option will be `c(1,2)`. These numbers refer to the lines where markers are located on the data file. Because the identification of the markers can be cumbersome, one should use the function `marker_type` to see their numbers, names and types:

```
marker_type(mark_all)

## Marker 1 ( M1 ) is of type B3.7
## Marker 2 ( M2 ) is of type D2.18
## Marker 3 ( M3 ) is of type D1.13
## Marker 4 ( M4 ) is of type A.4
## Marker 5 ( M5 ) is of type D2.18
## Marker 6 ( M6 ) is of type B3.7
## Marker 7 ( M7 ) is of type D2.15
## Marker 8 ( M8 ) is of type B3.7
## Marker 9 ( M9 ) is of type D1.10
## Marker 10 ( M10 ) is of type D2.17
## Marker 11 ( M11 ) is of type D2.16
## Marker 12 ( M12 ) is of type A.2
## Marker 13 ( M13 ) is of type C.8
## Marker 14 ( M14 ) is of type A.4
## Marker 15 ( M15 ) is of type A.4
## Marker 16 ( M16 ) is of type D2.17
## Marker 17 ( M17 ) is of type B2.6
```

```
## Marker 18 ( M18 ) is of type A.1
## Marker 19 ( M19 ) is of type B1.5
## Marker 20 ( M20 ) is of type A.1
## Marker 21 ( M21 ) is of type D2.16
## Marker 22 ( M22 ) is of type D1.10
## Marker 23 ( M23 ) is of type C.8
## Marker 24 ( M24 ) is of type B3.7
## Marker 25 ( M25 ) is of type B2.6
## Marker 26 ( M26 ) is of type A.1
## Marker 27 ( M27 ) is of type D1.12
## Marker 28 ( M28 ) is of type A.4
## Marker 29 ( M29 ) is of type D1.13
## Marker 30 ( M30 ) is of type B3.7
```

2. The grouping step is very simple and can be done by using the function `group`:

```
LGs <- group(mark_all)

## Selecting markers:
## group      1
## .....
## group      2
## .....
## group      3
## .....
```

For this function, optional arguments are `LOD` and `max.rf`, which define thresholds to be used when assigning markers to linkage groups. If none provided (default), criteria previously defined for the object `twopts` are used.

3. The previous command generates an object of class `group` and the command `print` for such object has two options. If you type:

LGs

you will get detailed information about the groups, that is, all linkage groups will be printed, displaying the names of markers in each one of them.

```
## This is an object of class 'group'
## It was generated from the object "mark_all"
##
## Criteria used to assign markers to groups:
##   LOD = 3 , Maximum recombination fraction = 0.4
##
## No. markers:          30
## No. groups:           3
## No. linked markers:   30
## No. unlinked markers: 0
##
## Printing groups:
## Group 1 : 15 markers
##   M1 M2 M3 M5 M6 M10 M11 M12 M14 M15 M17 M25 M26 M28 M30
##
## Group 2 : 10 markers
##   M4 M9 M16 M19 M20 M21 M23 M24 M27 M29
##
## Group 3 : 5 markers
##   M7 M8 M13 M18 M22
```

However, in case you just want to see some basic information (such as the number of groups, number of linked markers, etc):

```
print(LGs, detailed = FALSE)

## This is an object of class 'group'
## It was generated from the object "mark_all"
##
## Criteria used to assign markers to groups:
```

```
##      LOD = 3 , Maximum recombination fraction = 0.4
##
##      No. markers:           30
##      No. groups:           3
##      No. linked markers:    30
##      No. unlinked markers:  0
```

4. You can notice that all markers are linked to some linkage group. If the LOD Score threshold is changed to a higher value, some markers are kept unassigned:

```
LGs <- group(mark_all, LOD = 6)

##      Selecting markers:
##      group      1
##      .....
##      group      2
##      .....
##      group      3
##      ....

LGs

##      This is an object of class 'group'
##      It was generated from the object "mark_all"
##
##      Criteria used to assign markers to groups:
##      LOD = 6 , Maximum recombination fraction = 0.4
##
##      No. markers:           30
##      No. groups:           3
##      No. linked markers:    27
##      No. unlinked markers:  3
##
##      Printing groups:
```

```
## Group 1 : 15 markers
##      M1 M2 M3 M5 M6 M10 M11 M12 M14 M15 M17 M25 M26 M28 M30
##
## Group 2 : 8 markers
##      M4 M9 M16 M19 M20 M21 M23 M27
##
## Group 3 : 4 markers
##      M8 M13 M18 M22
```

5. Changing back to the previous criteria, now setting the maximum recombination fraction to 0.40:

```
LGs <- group(mark_all, LOD = 3, max.rf = 0.4)

##      Selecting markers:
##      group      1
##      .....
##      group      2
##      .....
##      group      3
##      .....

LGs

##      This is an object of class 'group'
##      It was generated from the object "mark_all"
##
##      Criteria used to assign markers to groups:
##      LOD = 3 , Maximum recombination fraction = 0.4
##
##      No. markers:          30
##      No. groups:           3
##      No. linked markers:   30
##      No. unlinked markers:  0
```

```
##
##   Printing groups:
##   Group 1 : 15 markers
##       M1 M2 M3 M5 M6 M10 M11 M12 M14 M15 M17 M25 M26 M28 M30
##
##   Group 2 : 10 markers
##       M4 M9 M16 M19 M20 M21 M23 M24 M27 M29
##
##   Group 3 : 5 markers
##       M7 M8 M13 M18 M22
```

4.5 Genetic mapping of linkage group 3

1. Once marker assignment to linkage groups is finished, the mapping step can take place. First of all, you must set the mapping function that should be used to display the genetic map throughout the analysis. You can choose between Kosambi or Haldane mapping functions. To use Haldane, type

```
set_map_fun(type = "haldane")
```

To use Kosambi

```
set_map_fun(type = "kosambi")
```

Now, you must define which linkage group will be mapped. In other words, a linkage group must be “extracted” from the object of class **group**, in order to be mapped. For simplicity, we will start here with the smallest one, which is linkage group 3. This can be easily done using the following code:

```
LG3 <- make_seq(LGs, 3)
```

The first argument (**LGs**) is an object of class **group** and the second is a number indicating which linkage group will be extracted, according to the results stored in object **LGs**. The object **LG3**, generated by function **make_seq**, is of class **sequence**, showing that this function can be used with several types of objects.

2. If you type

```
LG3
```

you will see which markers are comprised in the sequence, and also that no parameters have been estimated.

```
##  
## Number of markers: 5  
## Markers in the sequence:  
## M7 M8 M13 M18 M22  
##  
## Parameters not estimated.
```

3. To order these markers, one can use a two-point based algorithm such as Seriation (Buetow and Chakravarti, 1987), Rapid Chain Delineation (Doerge, 1996), Recombination Counting and Ordering (Van Os et al., 2005) and Unidirectional Growth (Tan and Fu, 2006):

```
LG3_ser <- seriation(LG3)  
LG3_rcd <- rcd(LG3)  
LG3_rec <- record(LG3)  
LG3_ug <- ug(LG3)
```

In this case, all algorithms provided the same results (results not shown).

4. To order by comparing all possible orders (exhaustive search), the function `compare` can be used:

```
LG3_comp <- compare(LG3)
```

This ordering step can take some time, depending on marker types in the linkage group. In the example, `LG3` contains one marker of type D1 and one of type D2, besides one marker segregating in 3:1 fashion (type C). Thus, although the number of possible orders is relatively small (60), for each order there are various possible combinations of linkage

phases. Also, the convergence of the EM algorithm takes considerably more time, because markers of type C are not very informative.

The first argument to the `compare` function is an object of class `sequence` (the extracted group LG3), and the object generated by this function is of class `compare`.

5. To see the results of the previous step, type

```
LG3_comp

##
## Number of orders: 50
## Best 30 unique orders          LOD      Nested LOD
## -----
## order  1:    22   7  13   8  18
##          CC  CC  RR  RR          0.00      0.00
##          CC  RC  RR  RR          -5.20     -5.20
## -----
## order  2:       7  22  13   8  18
##          CC  CC  RR  RR          0.00      0.00
##          CC  CR  RR  RR          -5.22     -5.22
## -----
## order  3:       7  18   8  13  22
##          CC  RR  RR  CC          -0.74      0.00
## -----
## order  4:       7  13   8  18  22
##          CC  RR  RR  CC          -0.93      0.00
## -----
## order  5:    22   7  18   8  13
##          CC  CC  RR  RR          -1.52      0.00
##          CC  RC  RR  RR          -5.18     -3.67
## -----
## order  6:       7  22  18   8  13
##          CC  CC  RR  RR          -1.52      0.00
##          CC  CR  RR  RR          -5.22     -3.71
## -----
```

```

## order 7:    22  7  8 13 18
##          CC  RR  RR  CC          -4.97      0.00
##          CC  CR  RR  CC          -8.99     -4.02
## -----
## order 8:     7 22  8 13 18
##          CC  RR  RR  CC          -4.97      0.00
##          CC  RC  RR  CC          -9.01     -4.04
## -----
## order 9:     7 18 13  8 22
##          CC  CC  RR  RC          -4.99      0.00
## -----
## order 10:    22  7 13 18  8
##          CC  CC  CC  RR          -5.03      0.00
##          CC  RC  CC  RR          -9.96     -4.93
## -----
## order 11:     7 22 13 18  8
##          CC  CC  CC  RR          -5.03      0.00
##          CC  CR  CC  RR          -9.99     -4.96
## -----
## order 12:     7  8 13 18 22
##          CR  RR  CC  CC          -5.22      0.00
## -----
## order 13:    22  7 18 13  8
##          CC  CC  CC  RR          -5.30      0.00
##          CC  RC  CC  RR          -8.97     -3.67
## -----
## order 14:     7 22 18 13  8
##          CC  CC  CC  RR          -5.30      0.00
##          CC  CR  CC  RR          -9.01     -3.71
## -----
## order 15:     7 13 18  8 22
##          CC  CC  RR  RC          -5.50      0.00
## -----

```

```

## order 16:      7   8  18  13  22
##           CR  RR  CC  CC           -5.75      0.00
## -----
## order 17:     22   7   8  18  13
##           CC  RR  RR  CC           -6.03      0.00
##           CC  CR  RR  CC           -9.97     -3.94
## -----
## order 18:      7  22   8  18  13
##           CC  RR  RR  CC           -6.03      0.00
##           CC  RC  RR  CC           -9.99     -3.96
## -----
## order 19:     18   8   7  13  22
##           RR  CR  RC  CC           -9.60      0.00
##           RR  CR  CC  CC          -13.87     -4.26
## -----
## order 20:      7  13  22   8  18
##           CC  CC  RR  RR           -9.85      0.00
##           CC  CC  RC  RR          -14.37     -4.51
## -----
## order 21:      8  18   7  13  22
##           RR  CC  CC  CC          -10.34      0.00
##           RR  CC  RC  CC          -13.62     -3.28
## -----
## order 22:      7  13  22  18   8
##           CC  CC  CC  RR          -10.40      0.00
##           CC  CC  CR  RR          -14.37     -3.97
## -----
## order 23:     18   8   7  22  13
##           RR  CR  RC  CC          -10.70      0.00
##           RR  CR  CC  CC          -14.65     -3.94
## -----
## order 24:     13   7  22   8  18
##           CC  CC  RR  RR          -10.70      0.00

```



```

## -----
## order 25:      8  18   7  22  13
##           RR  CC  CC  CC           -10.96      0.00
##           RR  CC  RC  CC           -14.62     -3.67
## -----
## order 26:     13   7  22  18   8
##           CC  CC  CC  RR           -10.96      0.00
## -----
## order 27:      7  18   8  22  13
##           CC  RR  RC  CR           -11.00      0.00
##           CC  RR  RC  CC           -14.13     -3.13
## -----
## order 28:      7   8  18  22  13
##           CR  RR  CC  CC           -11.51      0.00
##           CR  RR  CC  CR           -14.23     -2.72
## -----
## order 29:     13   7   8  18  22
##           CC  RR  RR  CC           -13.09      0.00
## -----
## order 30:     13   7  18   8  22
##           CC  CC  RR  RC           -13.60      0.00
## -----

```

Remember that for outcrossing populations, one needs to estimate marker order and also linkage phases between markers for a given order. However, because two point analysis provides information about linkage phases, this information is taken into consideration in the `compare` function, reducing the number of combinations to be evaluated. If a given linkage phase has LOD greater than 0.005 in the two point analysis, we assume that this phase is very unlikely and so does not need to be evaluated in the multipoint procedure used by `compare`. We did extensive simulations which showed that this is a good procedure.

By default, *OneMap* stores 50 orders, which may or may not be unique. The value of LOD refers to the overall LOD Score, considering all orders tested. **Nested** LOD refers to LOD Scores *within* a given order, that is, scores for different combinations of linkage phases for

the same marker order.

For example, order 1 has the largest value of log-likelihood and, therefore, its LOD Score is zero for a given combination of linkage phases (CC, CC, RR, RR). For this same order and other linkage phases, LOD Score is -5.20. Analyzing the results for order 2, notice that its highest LOD Score is very close to zero, indicating that this order is also quite plausible. Notice also that **Nested LOD** will always contain at least one zero value, corresponding to the best combination of phases for markers in a given order. Due to the information provided by two-point analysis, not all combinations are tested and that is the reason why the number of Nested LOD values is different for each order.

6. Unless one has some biological information, it is a good idea to choose the order with the highest likelihood. The final map can then be obtained with the command

```
LG3_final <- make_seq(LG3_comp, 1, 1)
```

The first argument is the object of class **compare**. The second argument indicates which order is chosen: 1 is for the order with highest likelihood, 2 is for the second best, and so on. The third argument indicates which combination of phases is chosen for a given order: 1 also means the combination with highest likelihood among all combinations of phases (based on Nested LOD).

For simplicity, these values are defaults, so typing

```
LG3_final <- make_seq(LG3_comp)
```

will have the same effect.

7. To see the final map type

```
LG3_final

##
## Printing map:
##
## Markers          Position          Parent 1          Parent 2
##
```

## 22 M22	0.00	a b	a a
## 7 M7	0.00	a a	a b
## 13 M13	36.15	a o	a o
## 8 M8	40.45	b a	b a
## 18 M18	46.32	a b	c d
##			
## 5 markers	log-likelihood: -318.8464		

At the leftmost position, marker names are displayed. **Position** shows the cumulative distance using the Kosambi mapping function. Finally, **Parent 1** and **Parent 2** show the diplotypes of both parents, that is, the combination in which alleles are arranged in the chromosomes, given the estimated linkage phase. Notation is the same as that used by Wu et al. (2002a). Details about how ordering algorithms can be chosen and used are presented by Mollinari et al. (2009).

4.6 Genetic mapping of linkage group 2

Now let us map the markers in linkage group number 2.

1. Again, “extract” that group from the object **LGs**:

```
LG2 <- make_seq(LGs, 2)
LG2

##
## Number of markers: 10
## Markers in the sequence:
## M4 M9 M16 M19 M20 M21 M23 M24 M27 M29
##
## Parameters not estimated.
```

Note that there are 10 markers in this group, so it is infeasible to use the **compare** function with all of them because it will take a very long time to proceed.

2. First, use **rcd** to get a preliminary order estimate:

```

LG2_rcd <- rcd(LG2)

##
## order obtained using RCD algorithm:
##
## 24 20 4 19 23 9 16 29 21 27
##
## calculating multipoint map using tol = 1e-04 .

LG2_rcd

##
## Printing map:
##
## Markers          Position          Parent 1      Parent 2
##
## 24 M24           0.00             a | | b      a | | b
## 20 M20           59.60             a | | b      d | | c
## 4 M4             72.37             a | | o      b | | o
## 19 M19           74.77             b | | a      o | | a
## 23 M23           87.59             a | | o      o | | a
## 9 M9            102.67             a | | b      a | | a
## 16 M16           123.02            a | | a      o | | b
## 29 M29           146.99            o | | a      o | | o
## 21 M21           157.46            o | | o      b | | a
## 27 M27           266.49            b | | o      a | | a
##
## 10 markers          log-likelihood: -674.3119

```

3. Use the `marker_type` function to check the segregation types of all markers in this group:

```
marker_type(LG2)

## Marker 4 ( M4 ) is of type A.4
## Marker 9 ( M9 ) is of type D1.10
## Marker 16 ( M16 ) is of type D2.17
## Marker 19 ( M19 ) is of type B1.5
## Marker 20 ( M20 ) is of type A.1
## Marker 21 ( M21 ) is of type D2.16
## Marker 23 ( M23 ) is of type C.8
## Marker 24 ( M24 ) is of type B3.7
## Marker 27 ( M27 ) is of type D1.12
## Marker 29 ( M29 ) is of type D1.13
```

4. Based on their segregation types and distribution on the preliminary map, markers M4, M23, M19, M20 and M24 are the most informative ones (type A is better, followed by type B). So, let us create a framework of ordered markers using `compare` for the most informative ones:

```
LG2_init <- make_seq(twoopts, c(4, 23, 19, 20, 24))
LG2_comp <- compare(LG2_init)
```

```
LG2_comp

##
## Number of orders: 50
## Best 35 unique orders                                LOD      Nested LOD
## -----
## order  1:    20   4  19  23  24
##          CR  RR  RC  CR          0.00      0.00
##          CR  RR  RC  RC         -5.52     -5.52
##          CR  RR  RR  CR        -10.25    -10.25
##          CR  RR  RR  RC        -22.09    -22.09
## -----
```

```

## order 2:    24  23  4  19  20
##          CR  CR  RR  RC          -4.02      0.00
##          RC  CR  RR  RC          -5.57     -1.55
## -----
## order 3:    20  4  23  19  24
##          CR  CR  RC  RR          -4.31      0.00
## -----
## order 4:    19  4  20  23  24
##          RR  CR  CC  CR          -4.77      0.00
##          RR  CR  CC  RC          -5.81     -1.04
## -----
## order 5:    23  19  4  20  24
##          RC  RR  CR  CR          -4.85      0.00
##          RR  RR  CR  CR          -21.43    -16.58
## -----
## order 6:    19  23  4  20  24
##          RC  CR  CR  CR          -4.86      0.00
## -----
## order 7:    23  4  19  20  24
##          CR  RR  RC  CR          -4.90      0.00
## -----
## order 8:    23  20  4  19  24
##          CC  CR  RR  RR          -5.15      0.00
## -----
## order 9:     4  19  20  23  24
##          RR  RC  CC  CR          -7.34      0.00
##          RR  RC  CC  RC          -8.37     -1.04
## -----
## order 10:   24  4  19  20  23
##          CC  RR  RC  CC          -7.41      0.00
## -----
## order 11:   20  4  19  24  23
##          CR  RR  RR  CR          -8.46      0.00

```

```

##          CR  RR  RR  RC          -11.26          -2.80
## -----
## order 12:      19   4  20  24  23
##          RR  CR  CR  CR          -9.90          0.00
##          RR  CR  CR  RC          -10.81          -0.91
## -----
## order 13:      23  24   4  19  20
##          CR  CC  RR  RC          -10.74          0.00
##          RC  CC  RR  RC          -13.83          -3.08
## -----
## order 14:       4  19  20  24  23
##          RR  RC  CR  CR          -12.47          0.00
##          RR  RC  CR  RC          -13.37          -0.91
## -----
## order 15:      19   4  23  20  24
##          RR  CR  CC  CR          -12.51          0.00
## -----
## order 16:      20  23   4  19  24
##          CC  CR  RR  RR          -12.53          0.00
## -----
## order 17:      24   4  19  23  20
##          CC  RR  RC  CC          -14.67          0.00
## -----
## order 18:      24   4  23  19  20
##          CC  CR  RC  RC          -14.69          0.00
## -----
## order 19:       4  19  23  20  24
##          RR  RC  CC  CR          -14.97          0.00
## -----
## order 20:       4  23  19  20  24
##          CR  RC  RC  CR          -14.98          0.00
## -----
## order 21:       4  20  19  23  24

```

```

##          CR  RC  RC  CR          -20.89          0.00
##          CR  RC  RC  RC          -22.10          -1.22
## -----
## order 22:      4  20  23  19  24
##          CR  CC  RC  RR          -20.89          0.00
## -----
## order 23:      24   4  20  19  23
##          CC  CR  RC  RC          -21.13          0.00
## -----
## order 24:      24   4  20  23  19
##          CC  CR  CC  RC          -21.13          0.00
## -----
## order 25:      24  23   4  20  19
##          CR  CR  CR  RC          -22.91          0.00
##          RC  CR  CR  RC          -24.46          -1.55
## -----
## order 26:      23   4  20  19  24
##          CR  CR  RC  RR          -23.80          0.00
## -----
## order 27:      23  19   4  24  20
##          RC  RR  CC  CR          -25.36          0.00
## -----
## order 28:      4  19  23  24  20
##          RR  RC  CR  CR          -25.71          0.00
##          RR  RC  RC  CR          -28.13          -2.42
## -----
## order 29:      19   4  23  24  20
##          RR  CR  CR  CR          -27.13          0.00
##          RR  CR  RC  CR          -29.81          -2.69
## -----
## order 30:      23   4  19  24  20
##          CR  RR  RR  CR          -27.66          0.00
## -----

```



```
## order 31:      19   4  24  23  20
##           RR  CC  CR  CC           -29.11           0.00
## -----
## order 32:       4  19  24  23  20
##           RR  RR  CR  CC           -29.41           0.00
## -----
## order 33:      19  23   4  24  20
##           RC  CR  CC  CR           -29.57           0.00
## -----
## order 34:      23  24   4  20  19
##           CR  CC  CR  RC           -29.64           0.00
## -----
## order 35:      19   4  24  20  23
##           RR  CC  CR  CC           -29.86           0.00
## -----
```

Now, the first argument to `make_seq` is an object of class `rf_2pts`, and the second argument is a vector of integers, specifying which molecular markers comprise the sequence.

5. Select the best order:

```
LG2_frame <- make_seq(LG2_comp)
```

6. Next, let us try to map the remaining markers, one at a time. Because there are more markers of type D1 than D2, the latter will be tried later. Starting with M9:

```
LG2_extend <- try_seq(LG2_frame, 9)

##  9 --> M9   : .....

LG2_extend

##
## LOD scores correspond to the best linkage phase combination
## for each position
```

```

##
## The symbol "*" outside the box indicates that more than one
## linkage phase is possible for the corresponding position
##
##
## Marker tested: 9
##
## Markers      LOD
## =====
## |              |
## |          -5.94 | 1
## | 20           |
## |          -15.82 | 2 *
## | 4            |
## |          -24.89 | 3 *
## | 19           |
## |          -4.21  | 4 *
## | 23           |
## |           0.00  | 5 *
## | 24           |
## |          -6.32  | 6
## |              |
## =====

```

Based on the LOD Scores, marker M9 is probably better located between markers M23 and M24. However, the “*” symbol indicates that more than one linkage phase is possible. Detailed results can be seen with

```

print(LG2_extend, 5)

##
## LOD is the overall LOD score (among all orders)
##
## NEST.LOD is the LOD score within the order

```

```
##
## Marker tested: 9
## -----
## |      |      |      |
## | 20   |      |      |
## |      | CR   | CR   |
## | 4    |      |      |
## |      | RR   | RR   |
## | 19   |      |      |
## |      | RC   | RC   |
## | 23   |      |      |
## |      | CC   | CC   |
## | 9    |      |      |
## |      | CR   | CC   |
## | 24   |      |      |
## |      |      |      |
## |-----|
## | LOD | 0.0 | -3.2 |
## |-----|
## | NEST. |      |      |
## | LOD | 0.0 | -3.2 |
## -----
```

The second argument indicates the position where to place the marker. Note that the first allele arrangement is the most likely one.

Also, we can obtain some useful diagnostic graphics using the argument `draw.try = TRUE` when using function `try_seq`:

```
LG2_extend <- try_seq(LG2_frame, 9, draw.try = TRUE)
```

The top figure represents the new genetic map obtained with the insertion of marker 9 between markers M23 and M24 (most likely placement). The left bottom figure represents the frame map M24 - M23 - M4 - M19 - M20 on the x-axis and the LOD Scores of the linkage maps obtained with the insertion of marker 9 at the beginning, between markers

and at the end of the frame map. The red triangle indicates the most likely position, where marker 9 is supposed to be placed. The right bottom figure is the recombination fraction matrix based on a color scale using the function `rf_graph_table`. See Section 4.9 for details. The diagnostic graphics show an almost monotonic recombination fraction matrix (the values are bigger as their distance from the diagonal increases). This pattern is typical of well ordered linkage groups. We can see that the position between markers M23 and M24 is the most likely one for positioning marker M9

7. Finally, the best order can be obtained with:

```
LG2_frame <- make_seq(LG2_extend, 5, 1)
```

When using `make_seq` with an object of class `try`, the second argument is the position on the map (according to the scale on the right of the output) and the last argument indicates linkage phases (defaults to 1, higher nested LOD).

It should be pointed out that the framework created by the function `compare` with (M20, M4, M19, M23 and M24) could be in reverse order (M24, M23, M19, M4 and M20) and still represent the same map. Thus, positioning of markers with the `try_seq` command can be different in your computer. For example, here marker M9 was better placed at position 5; however, if you obtain a reversed order, marker M9 would be better placed at position 2. In both cases the best position is between markers M24 and M23.

Adding other markers, one by one (output not shown):

```
LG2_extend <- try_seq(LG2_frame, 29)
LG2_frame <- make_seq(LG2_extend, 7)
LG2_extend <- try_seq(LG2_frame, 27)
LG2_frame <- make_seq(LG2_extend, 1)
LG2_extend <- try_seq(LG2_frame, 16)
LG2_frame <- make_seq(LG2_extend, 2)
LG2_extend <- try_seq(LG2_frame, 21)
LG2_final <- make_seq(LG2_extend, 6)
```

8. The process of adding markers sequentially can be automated with the use of function `order_seq`.

```

LG2_ord <- order_seq(LG2, n.init = 5, THRES = 3, draw.try = TRUE, wait = 1)

##
## Cross type: outcross
## Using segregation types of the markers to choose initial subset
##
## Comparing 60 orders:
##
##
|
|
|
|
|
|=
|
|==
|
|==
|
|==
|
|==
|
|===
|
|====
|
|====
|
|====
|
|=====
|
|=====
|

```

	0%
	2%
	3%
	5%
	7%
	8%
	10%
	12%
	13%
	15%
	17%
	18%





=====	75%
=====	77%
=====	78%
=====	80%
=====	82%
=====	83%
=====	85%
=====	87%
=====	88%
=====	90%
=====	92%
=====	93%
=====	95%
=====	97%
=====	98%
=====	100%
##	
##	


```

##
## Running try algorithm
## 9 --> M9 : .....
## 27 --> M27 : .....
## 29 --> M29 : .....
## 16 --> M16 : .....
## 21 --> M21 : .....
##
## LOD threshold = 3
##
## Positioned markers: 27 20 4 19 21 23 9 24
##
## Markers not placed on the map: 16 29
##
##
## Calculating LOD-Scores
## 16 --> M16 : .....
## 29 --> M29 : .....
##
##
## Placing remaining marker(s) at most likely position
## 29 --> M29 : .....
## 16 --> M16 : .....
##
## Estimating final genetic map using tol = 10E-5.

```

Basically, this function automates what the `try_seq` function does, using some pre-defined rules. In the function, `n.init = 5` means that five markers (the most informative ones) will be used in the `compare` step; `THRES = 3` indicates that the `try_seq` step will only add markers to the sequence which can be mapped with LOD Score greater than 3; `draw.try = TRUE` will display a diagnostic graphic for each `try_seq` step; `wait = 1` indicates the minimum time interval in seconds to display the diagnostic graphic.

NOTE: Although very useful, this function can be misleading, specially if there are not many fully informative markers, so use it carefully. Results can vary between multiple

runs on the same markers, of course.

9. Check the final order:

```
LG2_ord

##
## Best sequence found.
## Printing map:
##
## Markers          Position          Parent 1      Parent 2
##
## 27 M27           0.00             b | | o      a | | a
## 20 M20           21.18            a | | b      c | | d
## 4 M4             33.95            a | | o      o | | b
## 19 M19           36.23            b | | a      a | | o
## 21 M21           47.29            o | | o      a | | b
## 23 M23           52.46            a | | o      a | | o
## 9 M9             67.22            a | | b      a | | a
## 24 M24           95.14            a | | b      b | | a
##
## 8 markers          log-likelihood: -492.0175
##
##
##
## The following markers could not be uniquely positioned.
## Printing most likely positions for each unpositioned marker:
##
## -----
## |      | 16 | 29 |
## |----|-----|-----|
## |      | ** |      |
## | 27 |      |      |
## |      | *** |      |
## | 20 |      |      |
```

```

## |   |   |   |
## |  4 |   |   |
## |   |   |   |
## | 19 |   |   |
## |   |   |   |
## | 21 |   |   |
## |   |   |   |
## | 23 |   |   |
## |   |   |   |
## |  9 |   |   |
## |   |   |   |
## | 24 |   |   |
## |   |   | *** |
## -----
##
## '***' indicates the most likely position(s) (LOD = 0.0)
##
## '**' indicates very likely positions (LOD > -1.0)
##
## '*' indicates likely positions (LOD > -2.0)

```

Note that markers 16 and 29 could not be safely mapped to a single position (LOD Score > THRES in absolute value). The output displays the “safe” order and the most likely positions for markers not mapped, where “***” indicates the most likely position and “*” corresponds to other plausible positions.

10. To get the safe order (*i.e.*, without markers 16 and 29), use

```
LG2_safe <- make_seq(LG2_ord, "safe")
```

and to get the order with all markers, use

```
LG2_all <- make_seq(LG2_ord, "force")
LG2_all
```

```
##
## Printing map:
##
## Markers          Position          Parent 1      Parent 2
##
## 27 M27           0.00             b |   | o      a |   | a
## 16 M16           0.00             a |   | a      b |   | o
## 20 M20           16.03            a |   | b      c |   | d
##  4 M4            28.80            a |   | o      o |   | b
## 19 M19           31.08            b |   | a      a |   | o
## 21 M21           42.16            o |   | o      a |   | b
## 23 M23           47.02            a |   | o      a |   | o
##  9 M9            63.37            a |   | b      a |   | a
## 24 M24           90.84            a |   | b      b |   | a
## 29 M29          113.22            o |   | a      o |   | o
##
## 10 markers          log-likelihood: -585.4475
```

Notice that, for this linkage group, the “forced” map obtained with `order_seq` is the same as that obtained with `compare` plus `try_seq`, but *this is not always the case*.

11. The `order_seq` function can also perform two rounds of the `try_seq` algorithm, first using `THRES` and then `THRES - 1` as threshold. This generally results in safe orders with more markers mapped, but may take longer to run. To do this use the `touchdown` option:

```
LG2_ord <- order_seq(LG2, n.init = 5, THRES = 3, touchdown = TRUE)

##
## Cross type: outcross
## Using segregation types of the markers to choose initial subset
##
## Comparing 60 orders:
##
##
|
```

		0%
		2%
=		3%
==		5%
==		7%
==		8%
===		10%
====		12%
====		13%
====		15%
=====		17%
=====		18%
=====		20%
=====		22%
=====		23%
=====		25%
=====		27%



=====	55%
=====	57%
=====	58%
=====	60%
=====	62%
=====	63%
=====	65%
=====	67%
=====	68%
=====	70%
=====	72%
=====	73%
=====	75%
=====	77%
=====	78%
=====	80%
=====	82%

```

|
|=====| 83%
|
|=====| 85%
|
|=====| 87%
|
|=====| 88%
|
|=====| 90%
|
|=====| 92%
|
|=====| 93%
|
|=====| 95%
|
|=====| 97%
|
|=====| 98%
|
|=====| 100%
##
##
##
## Running try algorithm
## 9 --> M9 : .....
## 27 --> M27 : .....
## 29 --> M29 : .....
## 16 --> M16 : .....
## 21 --> M21 : .....
##
## LOD threshold = 3

```



```

##
## Positioned markers: 27 20 4 19 21 23 9 24
##
## Markers not placed on the map: 16 29
##
##
##
## Trying to map remaining markers with LOD threshold 2
## 16 --> M16 : .....
## 29 --> M29 : .....
##
## LOD threshold = 2
##
## Positioned markers: 27 20 4 19 21 23 9 24 29
##
## Markers not placed on the map: 16
##
##
## Calculating LOD-Scores
## 16 --> M16 : .....
##
##
## Placing remaining marker(s) at most likely position
## 16 --> M16 : .....
##
## Estimating final genetic map using tol = 10E-5.

LG2_ord

##
## Best sequence found.
## Printing map:
##
## Markers          Position          Parent 1          Parent 2

```

```

##
## 27 M27          0.00          b | | o      a | | a
## 20 M20          21.18         a | | b      c | | d
##  4 M4           33.95         a | | o      o | | b
## 19 M19          36.23         b | | a      a | | o
## 21 M21          47.32         o | | o      a | | b
## 23 M23          52.18         a | | o      a | | o
##  9 M9           68.53         a | | b      a | | a
## 24 M24          95.99         a | | b      b | | a
## 29 M29         118.37         o | | a      o | | o
##
## 9 markers          log-likelihood: -549.2296
##
##
##
## The following markers could not be uniquely positioned.
## Printing most likely positions for each unpositioned marker:
##
## -----
## |      | 16 |
## |----|-----|
## |      | ** |
## | 27 |      |
## |      | *** |
## | 20 |      |
## |      |      |
## |  4 |      |
## |      |      |
## | 19 |      |
## |      |      |
## | 21 |      |
## |      |      |
## | 23 |      |

```

```

## |   |   |
## | 9 |   |
## |   |   |
## | 24 |   |
## |   |   |
## | 29 |   |
## |   |   |
## -----
##
## '***' indicates the most likely position(s) (LOD = 0.0)
##
## '**' indicates very likely positions (LOD > -1.0)
##
## '*' indicates likely positions (LOD > -2.0)

```

For this particular sequence, the **touchdown** step could map marker M29, but this depends on the specific dataset.

12. Finally, to check for alternative orders (because we did not use exhaustive search), use the **ripple_seq** function:

```

ripple_seq(LG2_all, ws = 4, LOD = 3)

## 27-16-20-4-|-19-...
##   Alternative orders:
##     27 16 20 4 19 ... :    0.00 ( linkage phases: 1 1 2 4 ... )
##     16 27 20 4 19 ... :   -0.02 ( linkage phases: 1 1 2 4 ... )
##
## ...-27-|-16-20-4-19-|-21-... OK
##
## ...-16-|-20-4-19-21-|-23-... OK
##
## ...-20-|-4-19-21-23-|-9-... OK
##

```

```
## ...-4-|-19-21-23-9-|-24-... OK
##
## ...-19-|-21-23-9-24-|-29-... OK
##
## 21-|-23-9-24-29
##   Alternative orders:
##   ... 21 23 9 24 29 :    0.00 ( linkage phases: ... 3 1 2 3 )
##   ... 21 23 9 29 24 :   -2.06 ( linkage phases: ... 3 1 4 3 )
```

We should do this to any of the orders we found, either using `try_seq` or `order_seq`. Here, we choose `LG2_all` for didactic purposes only. The second argument, `ws = 4`, means that subsets (windows) of four markers will be permuted sequentially (4! orders for each window), to search for other plausible orders. The `LOD` argument means that only orders with `LOD` Score smaller than 3 will be printed.

The output shows sequences of four numbers, because `ws = 4`. They are followed by an `OK` if there is no alternative order with `LOD` Score smaller than `LOD = 3` in absolute value, or by a list of alternative orders. In the example, the first and last sequences showed alternative orders with `LOD` smaller than `LOD = 3`. However, the best order was the original one (`LOD = 0.00`).

If there were an alternative order more likely than the original, one should check the difference between these orders (and linkage phases) and change it using, for example, functions `drop_marker` (see Section 4.8) and `try_seq`, or by typing the new order. You can use `$seq.num` and `$seq.phases` after the name of the sequence (*e.g.*, `LG2_all$seq.num` and `LG2_all$seq.phases`) to obtain the original order and linkage phases, make the necessary changes (by copying and pasting) and then use the function `map` (see Section 4.8) to reestimate the genetic map for the new order.

Here, the `ripple_seq` function showed that the final order obtained is indeed the best for this linkage group. The map can then be printed using

```
LG2_all

##
## Printing map:
```

```
##
## Markers          Position          Parent 1      Parent 2
##
## 27 M27           0.00             b | | o      a | | a
## 16 M16           0.00             a | | a      b | | o
## 20 M20           16.03            a | | b      c | | d
##  4 M4            28.80            a | | o      o | | b
## 19 M19           31.08            b | | a      a | | o
## 21 M21           42.16            o | | o      a | | b
## 23 M23           47.02            a | | o      a | | o
##  9 M9            63.37            a | | b      a | | a
## 24 M24           90.84            a | | b      b | | a
## 29 M29          113.22            o | | a      o | | o
##
## 10 markers          log-likelihood: -585.4475
```

4.7 Genetic mapping of linkage group 1

1. Finally, linkage group 1 (the largest one) will be analyzed. Extract markers:

```
LG1 <- make_seq(LGs, 1)
```

2. Construct the linkage map, by automatically using the try algorithm:

```
LG1_ord <- order_seq(LG1, n.init = 6, touchdown = TRUE)

##
## Cross type: outcross
## Using segregation types of the markers to choose initial subset
## WARNING: this operation may take a VERY long time
##
## Comparing 360 orders:
##
##
```

	0%
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	2%
=	2%
=	3%
=	4%
=	5%
==	5%
==	6%
==	7%
==	8%
===	9%
===	10%
===	11%
====	12%
====	13%

=====	14%
=====	15%
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	=====	88%
	=====	89%
	=====	90%
	=====	91%
	=====	92%
	=====	93%
	=====	94%
	=====	95%
	=====	95%

```

|=====| 96%
|
|=====| 97%
|
|=====| 98%
|
|=====| 98%
|
|=====| 99%
|
|=====| 100%
##
##
##
## Running try algorithm
## 25 --> M25 : .....
## 1 --> M1 : .....
## 6 --> M6 : .....
## 30 --> M30 : .....
## 2 --> M2 : .....
## 5 --> M5 : .....
## 10 --> M10 : .....
## 11 --> M11 : .....
## 3 --> M3 : .....
##
## LOD threshold = 3
##
## Positioned markers: 12 3 14 2 1 10 17 28 26 6 15 11
##
## Markers not placed on the map: 5 25 30
##
##
##

```

```

## Trying to map remaining markers with LOD threshold 2
## 5 --> M5 : .....
## 25 --> M25 : .....
## 30 --> M30 : .....
##
## LOD threshold = 2
##
## Positioned markers: 12 3 14 2 1 10 17 28 26 6 15 5 25 11
##
## Markers not placed on the map: 30
##
##
## Calculating LOD-Scores
## 30 --> M30 : .....
##
##
## Placing remaining marker(s) at most likely position
## 30 --> M30 : .....
##
## Estimating final genetic map using tol = 10E-5.

LG1_ord

##
## Best sequence found.
## Printing map:
##
## Markers          Position          Parent 1          Parent 2
##
## 12 M12           0.00             a | | b          a | | c
## 3 M3              19.57            a | | o          o | | o
## 14 M14           32.63            o | | a          o | | b
## 2 M2              39.79            o | | o          a | | o
## 1 M1              60.61            a | | b          a | | b

```

```

## 10 M10          66.72          a | | a      b | | o
## 17 M17          81.51          o | | a      b | | a
## 28 M28          82.87          o | | a      o | | b
## 26 M26         115.24          b | | a      c | | d
##  6 M6           124.26          a | | b      a | | b
## 15 M15          156.63          a | | o      b | | o
##  5 M5           162.10          o | | o      a | | o
## 25 M25          174.43          a | | o      b | | a
## 11 M11          184.57          o | | o      a | | b
##
## 14 markers          log-likelihood: -938.2285
##
##
##
## The following markers could not be uniquely positioned.
## Printing most likely positions for each unpositioned marker:
##
## -----
## |      | 30 |
## |----|-----|
## |      | *** |
## | 12 |      |
## |      | ** |
## |  3 |      |
## |      |      |
## | 14 |      |
## |      |      |
## |  2 |      |
## |      |      |
## |  1 |      |
## |      |      |
## | 10 |      |
## |      |      |

```

```

## | 17 |      |
## |    |      |
## | 28 |      |
## |    |      |
## | 26 |      |
## |    |      |
## |  6 |      |
## |    |      |
## | 15 |      |
## |    |      |
## |  5 |      |
## |    |      |
## | 25 |      |
## |    |      |
## | 11 |      |
## |    |      |
## -----
##
## '***' indicates the most likely position(s) (LOD = 0.0)
##
## '**' indicates very likely positions (LOD > -1.0)
##
## '*' indicates likely positions (LOD > -2.0)

```

Notice that the second round of `try_seq` added markers M5 and M25.

3. Now, get the order with all markers:

```

(LG1_final <- make_seq(LG1_ord, "force"))

##
## Printing map:
##
## Markers          Position          Parent 1      Parent 2

```



```
##
## 30 M30          0.00          a | | b      a | | b
## 12 M12          1.00          b | | a      c | | a
##  3 M3           20.57         o | | a      o | | o
## 14 M14          33.63         a | | o      b | | o
##  2 M2           40.79         o | | o      o | | a
##  1 M1           61.61         b | | a      b | | a
## 10 M10          67.72         a | | a      o | | b
## 17 M17          82.51         a | | o      a | | b
## 28 M28          83.87         a | | o      b | | o
## 26 M26         116.25         a | | b      d | | c
##  6 M6           125.26         b | | a      b | | a
## 15 M15          157.63         o | | a      o | | b
##  5 M5           163.10         o | | o      o | | a
## 25 M25          175.44         o | | a      a | | b
## 11 M11          185.57         o | | o      b | | a
##
## 15 markers          log-likelihood: -948.0381
```

4. Check the final map:

```
ripple_seq(LG1_final)

## 30-12-3-14-|-2-...
##   Alternative orders:
##   30 12 3 14 2 ... :    0.00 ( linkage phases: 4 1 4 2 ... )
##   12 30 3 14 2 ... :   -0.20 ( linkage phases: 4 4 4 2 ... )
##
## ...-30-|-12-3-14-2-|-1-... OK
##
## ...-12-|-3-14-2-1-|-10-... OK
##
## ...-3-|-14-2-1-10-|-17-... OK
##
```

```

## ...-14-|-2-1-10-17-|-28-... OK
##
## ...-2-|-1-10-17-28-|-26-...
##   Alternative orders:
##   ... 2 1 10 17 28 26 ... :    0.00 ( linkage phases: ... 3 1 4 1 2 ... )
##   ... 2 1 10 28 17 26 ... :   -2.33 ( linkage phases: ... 3 1 4 1 2 ... )
##
## ...-1-|-10-17-28-26-|-6-...
##   Alternative orders:
##   ... 1 10 17 28 26 6 ... :    0.00 ( linkage phases: ... 1 4 1 2 3 ... )
##   ... 1 10 28 17 26 6 ... :   -2.33 ( linkage phases: ... 1 4 1 2 3 ... )
##
## ...-10-|-17-28-26-6-|-15-...
##   Alternative orders:
##   ... 10 17 28 26 6 15 ... :    0.00 ( linkage phases: ... 4 1 2 3 1 ... )
##   ... 10 28 17 26 6 15 ... :   -2.33 ( linkage phases: ... 4 1 2 3 1 ... )
##
## ...-17-|-28-26-6-15-|-5-... OK
##
## ...-28-|-26-6-15-5-|-25-... OK
##
## ...-26-|-6-15-5-25-|-11-... OK
##
## 6-|-15-5-25-11 OK

```

No better order was observed.

5. Print it

```

LG1_final

##
## Printing map:
##
## Markers          Position          Parent 1          Parent 2

```

```
##
## 30 M30          0.00      a | | b      a | | b
## 12 M12          1.00      b | | a      c | | a
##  3 M3           20.57     o | | a      o | | o
## 14 M14          33.63     a | | o      b | | o
##  2 M2           40.79     o | | o      o | | a
##  1 M1           61.61     b | | a      b | | a
## 10 M10          67.72     a | | a      o | | b
## 17 M17          82.51     a | | o      a | | b
## 28 M28          83.87     a | | o      b | | o
## 26 M26         116.25     a | | b      d | | c
##  6 M6           125.26     b | | a      b | | a
## 15 M15          157.63     o | | a      o | | b
##  5 M5           163.10     o | | o      o | | a
## 25 M25          175.44     o | | a      a | | b
## 11 M11          185.57     o | | o      b | | a
##
## 15 markers      log-likelihood: -948.0381
```

6. As an option, different algorithms to order markers could be applied:

```
LG1_ser <- seriation(LG1)
LG1_rcd <- rcd(LG1)
LG1_rec <- record(LG1)
LG1_ug <- ug(LG1)
```

There are some differences between the results. Seriation did not provide good results in this case. See Mollinari et al. (2009) for an evaluation of these methods.

4.8 Map estimation for an arbitrary order

1. If, for any reason, one wants to estimate parameters for a given linkage map (*e.g.*, for other orders on published papers), it is possible to define a sequence and use the `map` function. For example, for markers M30, M12, M3, M14 and M2, in this order, use

```
any_seq <- make_seq(twopts, c(30, 12, 3, 14, 2))
(any_seq_map <- map(any_seq))

##
## Printing map:
##
## Markers          Position          Parent 1          Parent 2
##
## 30 M30           0.00             a | | b          a | | b
## 12 M12           1.00             b | | a          c | | a
## 3 M3             20.57            o | | a          o | | o
## 14 M14           33.63            a | | o          b | | o
## 2 M2             41.70            o | | o          o | | a
##
## 5 markers          log-likelihood: -320.9012
```

This is a subset of the first linkage group. When used this way, the `map` function searches for the best combination of phases between markers and prints the results.

2. Furthermore, a sequence can also have user-defined linkage phases. The next example shows (incorrect) phases used for the same order of markers:

```
any_seq <- make_seq(twopts, c(30, 12, 3, 14, 2), phase = c(4, 1, 4, 3))
(any_seq_map <- map(any_seq))

##
## Printing map:
##
## Markers          Position          Parent 1          Parent 2
##
## 30 M30           0.00             a | | b          a | | b
## 12 M12           1.00             b | | a          c | | a
## 3 M3             20.57            o | | a          o | | o
## 14 M14           33.63            a | | o          b | | o
## 2 M2            379.02            o | | o          a | | o
```

```
##  
## 5 markers                log-likelihood: -362.3389
```

3. If one needs to add or drop markers from a predefined sequence, functions `add_marker` and `drop_marker` can be used. For example, to add markers 4 to 8 to `any_seq`

```
(any_seq <- add_marker(any_seq, 4:8))  
  
##  
## Number of markers: 10  
## Markers in the sequence:  
## M30 M12 M3 M14 M2 M4 M5 M6 M7 M8  
##  
## Parameters not estimated.
```

Removing markers 3, 4, 5, 12 and 30 from `any_seq`:

```
(any_seq <- drop_marker(any_seq, c(3, 4, 5, 12, 30)))  
  
##  
## Number of markers: 5  
## Markers in the sequence:  
## M14 M2 M6 M7 M8  
##  
## Parameters not estimated.
```

After that, the map needs to be re-estimated.

4.9 Plotting the recombination fraction matrix

For a given sequence, it is possible to plot the recombination fraction matrix and LOD Scores based on a color scale using the function `rf_graph_table`. This matrix can provide some useful diagnostics about the map.

1. For example, using the function `group` with `LOD = 2.5`:

```
(LGs <- group(mark_all, LOD = 2.5))
```

Due to the small value used for the LOD Score (2.5, not adequate and resulting in false positives), markers from different groups were placed together.

2. Ordering markers (results not shown):

```
LG_err <- make_seq(LGs, 2)
LG_err_ord <- order_seq(LG_err)
```

The map using option “force”:

```
(LG_err_map <- make_seq(LG_err_ord, "force"))
```

```
##
## Printing map:
##
## Markers          Position          Parent 1          Parent 2
##
## 27 M27           0.00             b | | o           a | | a
## 16 M16           0.00             a | | a           b | | o
## 20 M20           16.03            a | | b           c | | d
##  4 M4            28.80            a | | o           o | | b
## 19 M19           31.08            b | | a           a | | o
## 21 M21           42.17            o | | o           a | | b
## 23 M23           47.02            a | | o           a | | o
##  9 M9            63.37            a | | b           a | | a
## 24 M24           90.74            a | | b           b | | a
## 29 M29          112.66            o | | a           o | | o
## 22 M22          190.64            a | | b           a | | a
##  7 M7            190.64            a | | a           a | | b
## 18 M18          235.00            a | | b           c | | d
```

```
## 8 M8                240.69          b | | a          b | | a
## 13 M13              244.82          a | | o          a | | o
##
## 15 markers          log-likelihood: -907.33
```

3. A careful examination of the results shows that there are problems on the map. This can be done by plotting the recombination fraction matrix:

```
rf_graph_table(LG_err_map)
```

The recombination fractions are plotted below the diagonal and the LOD Scores are plotted above the diagonal. The color scale varies from red (small distances or big LODs) to dark blue. This color scale follows the “rainbow” color palette with **start** argument equal to 0 and **end** argument equal to 0.65. White cells indicate combinations of markers for which the recombination fractions cannot be estimated (D_1 and D_2).

Clicking on the cell corresponding to two markers (off the secondary diagonal), you can see some information about them. For example, clicking on the cell corresponding to markers **M4** and **M19** you can see their names, types (**A.4** and **B1.5**), recombination fraction (**rf** = 0.02281) and LOD Scores for each possible linkage phase. Clicking on a cell on the diagonal, some information about the corresponding marker is shown, including percent of missing data. This is quite useful in helping to interpret the results.

Looking at the matrix, it is possible to see two groups: one with markers from **LG2** (**M27**, **M16**, **M20**, **M4**, **M19**, **M21**, **M23**, **M9**, **M24**, and **M29**) and other with markers from **LG3** (**M22**, **M7**, **M18**, **M8** and **M13**). There is a big gap between markers **M22** and **M29** (**rf** = 0.4594). At this position, the group should be divided, that is, a higher LOD Score should be used. Notice that these two groups were placed together due to a false linkage (false positive) detected between markers **M4** and **M22** (LOD Score 2.9), because of not using an appropriate LOD threshold (a more conservative value).

The **rf_graph_table** can also be used to check the order of markers based on the monotonicity of the matrix: as we get away from the secondary diagonal, the recombination fraction values should increase. For another example of function **rf_graph_table**, see Section 5.9.

4.10 Drawing the genetic map

1. Once all linkage groups were obtained, we can draw a simple map using the function `draw_map`. We can draw a genetic map for all linkage groups:

```
maps <- list(LG1_final, LG2_final, LG3_final)
draw_map(maps, names = TRUE, grid = TRUE, cex.mrk = 0.7)
```

2. For a specific linkage group:

```
draw_map(LG1_final, names = TRUE, grid = TRUE, cex.mrk = 0.7)
```

It is obvious that function `draw_map` draws a very simple graphic representation of the genetic map. But once the distances and the linkage phases are estimated, better map figures can be drawn by the user with any appropriate software. There are several free software that can be used, such as MapChart (Voorrips, 2002).

5 F₂ example

Starting in version 2.0-0, *OneMap* can also deal with inbred-based populations (F₂, backcrosses and RILs). In this section we explain how to proceed with the analysis in an F₂ population. This procedure can be used for backcrosses and RILs as well. If you are not familiar with R software, we recommend reading Section 2. Most of the steps for constructing an F₂ genetic map are the same as those used in the outcrossing example, thus details can be obtained on Section 4, However, this section can be read alone.

5.1 Creating the data file

For F₂, backcrosses and RILs, two input formats are accepted. The user can choose between the standard *OneMap* file format (Section 4.1) or the same raw file used by MAPMAKER/EXP (Lander et al., 1987). Therefore, one should have no difficulty in using data sets already available for MAPMAKER/EXP. This type of raw file can contain phenotypic information, but this will not be used during map construction. This file, combined with the map file produced by *OneMap*, can be readily used for QTL mapping using *R/qtl* (Broman et al., 2008) or *QTL Cartographer* (Wang et al., 2010), among others. Here, we briefly present how to set

up this data file. For more detailed information see the MAPMAKER/EXP manual (Lincoln et al., 1993).

The first line of your data file should be:

```
data type xxxx
```

where **xxxx** is one of the following data types:

f2 backcross	for backcrosses
f2 intercross	for F_2
ri self	for RILs by selfing
ri sib	for RILs by sib mating

The second line should contain the number of individuals in the progeny, the number of markers and the number of quantitative traits. Then, the genotype information is included for each marker. The character “*” indicates the beginning of information of a marker, followed by the marker name. The codification for genotypes is the following:

A:	homozygous for allele A (from parent 1 - AA)
B:	homozygous for allele B (from parent 2 - BB)
H:	heterozygous carrying both alleles (AB)
C:	Not homozygous for allele A (Not AA)
D:	Not homozygous for allele B (Not BB)
-:	Missing data for the individual at this marker

The “symbols” option, used in MAPMAKER/EXP files, is also accepted (please, see the manual).

The quantitative trait data should come after the genotypic data and has a similar format, except the trait values for each individual must be separated by at least one space, a tab or a line break. A dash (-) indicates missing data. Here is an example of such a file for an F_2 population with 10 individuals, 5 markers and 2 quantitative traits:

```
data type f2 intercross
```

```
10 5 2
```

```
*M1 A B H H A - B A A B
```

```

*M2 C - C C C - - C C A
*M3 D B D D - - B D D B
*M4 C C C - A C C A A C
*M5 C C C C C C C C C C

*weight 10.2 - 9.4 11.3 11.9 8.9 - 11.2 7.8 8.1
*length 1.7 2.1 - 1.8 2.0 1.0 - 1.7 1.0 1.1

```

This file must be saved in plain text format using a simple text editor such as *notepad*. Historically, MAPMAKER/EXP uses the “.raw” extension for this file, however, you can use other extensions, for example, “.txt”. If you want to see an example of how this file should be, you can open “fake_bc_onemap.raw” and “fake_f2_onemap.raw”, both available with *OneMap* and saved in the directory you installed it (use `system.file(package = "onemap")` to see where it is).

Now, let us load *OneMap*:

1. Start R by double-clicking its icon.
2. Load *OneMap* (after installing it; for details see Sections 2.4 and 3):

```
library(onemap)
```

3. To save your project anytime, type:

```
save.image("C:/.../yourfile.RData")
```

specifying the file path, or access the toolbar File → Save Workspace.

5.2 Importing data

1. Once you created your data file, you can use the function `read_mapmaker` to import it to *OneMap*.

```
fake_f2_onemap <- read_mapmaker(dir = "C:/workingdirectory",
                                file = "your_data_file.raw")
```

The first argument is the directory where the input file is located, so modify it accordingly. The second one is the data file name. In this example, an object named `fake_f2_onemap` was created. Notice that if you leave the argument `dir` blank, the file will be read from your current *working directory*. To set a working directory, see Section 2.5.

2. For this example, we will use a simulated data set from an F_2 population which is distributed along with the *OneMap* package. Because this particular data set is distributed along with the package, you can load it typing

```
data(fake_f2_onemap)
fake_f2_onemap

## This is an object of class 'onemap'
##   Type of cross:      f2
##   No. individuals:   200
##   No. markers:       66
##   CHROM information: no
##   POS information:   no
##   Percent genotyped: 85
##
## Segregation types:
##   AA : AB : BB --> 36
##   Not AA : AA --> 15
##   Not BB : BB --> 15
##
##   No. traits:        1
##   Missing trait values:
##   Trait_1: 0
```

The data consists of a sample of 200 individuals genotyped for 66 markers (36 co-dominant (AA, AB or BB), 15 dominant (Not AA or AA) and 15 dominant (Not BB or BB) with 15% of missing data. You can also see that there is phenotypic information for one trait in the data set.

5.3 Estimating two-point recombination fractions

1. Let us start the analysis by estimating the recombination fraction between all pairs of markers using two-point tests:

```
twopts_f2 <- rf_2pts(fake_f2_onemap)
```

There are two optional arguments in function `rf_2pts`: `LOD` and `max.rf` which indicate the minimum LOD Score and the maximum recombination fraction to declare linkage (they default to 3.0 and 0.5, respectively).

2. If you want to see the results for any given pair of markers, say M12 and M42, use:

```
print(twopts_f2, c("M12", "M42"))

## Results of the 2-point analysis for markers: M12 and M42
## Criteria: LOD = 3 , Maximum recombination fraction = 0.5
##
##          rf          LOD
## 0.3575533 1.9430940
```

5.4 Assigning markers to linkage groups

1. To assign markers to linkage groups, first use the function `make_seq` to create a sequence with all markers:

```
mark_all_f2 <- make_seq(twopts_f2, "all")
```

The function `make_seq` is used to create sequences from objects of several kinds. Here, the first argument is of class `rf_2pts` and the second argument specifies which markers one wants to use ("`all`" indicates that all markers will be analyzed). To subset markers, say M1, M3 and M7, use:

```
mrk_subset <- make_seq(twopts_f2, c(1, 3, 7))
```

2. You can assign markers to linkage groups using the function `group`:

```
(LGs_f2 <- group(mark_all_f2, LOD = 3, max.rf = 0.5))

##      Selecting markers:
##      group      1
##      .....
##      group      2
##      .....
##      group      3
##      .....
##      This is an object of class 'group'
##      It was generated from the object "mark_all_f2"
##
##      Criteria used to assign markers to groups:
##      LOD = 3 , Maximum recombination fraction = 0.5
##
##      No. markers:          66
##      No. groups:           3
##      No. linked markers:   66
##      No. unlinked markers:  0
##
##      Printing groups:
##      Group 1 : 27 markers
##      M1 M3 M4 M6 M7 M9 M12 M13 M17 M23 M26 M27 M29 M30 M31 M34 M35 M36 M40 M42 M44
##
##      Group 2 : 16 markers
##      M2 M5 M8 M10 M11 M25 M32 M33 M37 M41 M43 M45 M51 M54 M61 M66
##
##      Group 3 : 23 markers
##      M14 M15 M16 M18 M19 M20 M21 M22 M24 M28 M38 M39 M47 M48 M49 M50 M52 M56 M57 M
```

The arguments `LOD` and `max.rf` define thresholds to be used when assigning markers to linkage groups. If none provided (default), criteria previously defined for the object

`twopts_f2` are used. We can see that the markers were assigned to three linkage groups with 27, 16 and 23 markers, with no unlinked markers.

5.5 Genetic mapping of linkage group 2

After assigning markers to linkage groups, the next step is to order the markers within each group.

1. First, let us choose the mapping function used to display the genetic map. We can choose between Kosambi or Haldane mapping functions. To use Haldane, type

```
set_map_fun(type = "haldane")
```

To use Kosambi

```
set_map_fun(type = "kosambi")
```

2. To define which linkage group will be mapped, we must “extract” it from the object of class `group`. Let us extract group 2 using:

```
LG2_f2 <- make_seq(LGs_f2, 2)
```

The first argument is an object of class `group` and the second is a number indicating which linkage group will be extracted. In this case, the object `LGs_f2`, generated by function `group`, is of class `group`, showing that this function can handle different classes of objects.

3. If you type

```
LG2_f2
```

you will see which markers are comprised in the sequence, and also that no parameters have been estimated.

```
##
## Number of markers: 16
## Markers in the sequence:
## M2 M5 M8 M10 M11 M25 M32 M33 M37 M41
## M43 M45 M51 M54 M61 M66
##
## Parameters not estimated.
```

4. To order these markers, one can use a two-point based algorithm such as Seriation (Buetow and Chakravarti, 1987), Rapid Chain Delineation (Doerge, 1996), Recombination Counting and Ordering (Van Os et al., 2005) and Unidirectional Growth (Tan and Fu, 2006):

```
LG2_ser_f2 <- seriation(LG2_f2)
LG2_rcd_f2 <- rcd(LG2_f2)
LG2_rec_f2 <- record(LG2_f2)
LG2_ug_f2 <- ug(LG2_f2)
```

For this particular data set, the algorithms provided different results (results not shown here). For an evaluation and comparison of these methods, see Mollinari et al. (2009).

Now, let us use a multipoint approach to order markers within group 2. We could use the following: for each possible order of this group, we calculate the multipoint likelihood, and then compare all of them, choosing the most likely one (highest likelihood). For a moderate number of markers (up to 10 or 11), this is feasible. This procedure is implemented in the function `compare`. Although feasible, with more than 7 markers the function `compare` could take a very long time, depending on the data set and computational resources used. A detailed use of this function can be seen in Section 4.5. It is important to say that we do not need to estimate the linkage phases for F_2 populations; therefore, we can use a slightly large number of markers in function `compare`. However, for 16 markers, which is the number of markers in group 2, the use of function `compare` is infeasible, and we should use another approach.

Thus we will apply the same procedure used in Section 4.6. We will choose a moderate number of markers, say 6, to create a framework using the function `compare` and then

position the remaining markers using the function `try_seq`. The way we choose these initial markers in inbred-based populations (F_2 , backcrosses and RILs) is somewhat different from outcrossing populations.

We recommend two methods: i) randomly choose a number of markers and calculate the multipoint likelihood of all possible orders (using the function `compare`). If the LOD Score of the second best order is greater than a given threshold, say 3, then take the best order to proceed with the next step. If not, repeat the procedure; ii) use some two-point based algorithm to construct a map; then, take equally spaced markers from this map. Then, create a framework of ordered markers using the function `compare`. Next, try to map the remaining markers, one at a time, beginning with co-dominant ones (most informative ones), then add the dominant ones. You can do this procedure manually, like shown in Section 4.6; this procedure is also automated in function `order_seq` which we will use here for the latter procedure:

```
LG2_f2_ord <- order_seq(input.seq = LG2_f2, n.init = 5,
                        subset.search = "twopt",
                        twopt.alg = "rcd", THRES = 3,
                        draw.try = TRUE, wait = 1)

##
## Cross type:  f2
## Choosing initial subset using 'two-point' approach
##
## order obtained using RCD algorithm:
##
##  41 10 43 11 2 32 45 54 66 61 25 5 51 33 37 8
##
## calculating multipoint map using tol = 0.1 .
##
##
## Comparing 60 orders:
##
##
|
```


		0%
		2%
=		3%
==		5%
==		7%
==		8%
===		10%
====		12%
====		13%
====		15%
=====		17%
=====		18%
=====		20%
=====		22%
=====		23%
=====		25%
=====		27%



=====	55%
=====	57%
=====	58%
=====	60%
=====	62%
=====	63%
=====	65%
=====	67%
=====	68%
=====	70%
=====	72%
=====	73%
=====	75%
=====	77%
=====	78%
=====	80%
=====	82%

```

|
|=====| 83%
|
|=====| 85%
|
|=====| 87%
|
|=====| 88%
|
|=====| 90%
|
|=====| 92%
|
|=====| 93%
|
|=====| 95%
|
|=====| 97%
|
|=====| 98%
|
|=====| 100%
##
##
##
## Running try algorithm
## 66 --> M66 : .....
## 37 --> M37 : .....
## 61 --> M61 : .....
## 10 --> M10 : .....
## 33 --> M33 : .....
## 25 --> M25 : .....
## 43 --> M43 : .....

```

```

## 32 --> M32 : .....
## 51 --> M51 : .....
## 45 --> M45 : .....
## 11 --> M11 : .....
##
## LOD threshold = 3
##
## Positioned markers: 41 10 2 43 32 54 66 25 5 51 33 37 8
##
## Markers not placed on the map: 11 45 61
##
##
## Calculating LOD-Scores
## 11 --> M11 : .....
## 45 --> M45 : .....
## 61 --> M61 : .....
##
##
## Placing remaining marker(s) at most likely position
## 61 --> M61 : .....
## 11 --> M11 : .....
## 45 --> M45 : .....
##
## Estimating final genetic map using tol = 10E-5.

```

The first argument is an object of class `sequence`. `n.init = 5` means that five markers will be used in the `compare` step. The argument `subset.search = "twopt"` indicates that these five markers should be chosen by using a two-point method, which will be Rapid Chain Delineation, as indicated by the argument `twopt.alg = "rcd"`. `THRES = 3` indicates that the `try_seq` step will only add markers to the sequence which can be mapped with LOD Score greater than 3. `draw.try = TRUE` will display a diagnostic graphic for each `try_seq` step (see Section 4.6). `wait = 1` indicates the time interval in seconds to display the diagnostic graphic. NOTE: Although very useful, this function can be misleading, specially if there is a considerable amount of missing data and dominant

markers, so use it carefully.

5. Check the final order:

```
LG2_f2_ord

##
## Best sequence found.
## Printing map:
##
## Markers          Position
##
## 41 M41           0.00
## 10 M10           8.66
##  2 M2            16.87
## 43 M43           22.27
## 32 M32           25.78
## 54 M54           33.28
## 66 M66           39.99
## 25 M25           47.15
##  5 M5            51.30
## 51 M51           55.63
## 33 M33           61.27
## 37 M37           65.58
##  8 M8            71.47
##
## 13 markers          log-likelihood: -942.961
##
##
##
## The following markers could not be uniquely positioned.
## Printing most likely positions for each unpositioned marker:
##
## -----
## |      | 11 | 45 | 61 |
```

```

## |----|-----|-----|-----|
## |    |    |    |    |
## | 41 |    |    |    |
## |    |    |    |    |
## | 10 |    |    |    |
## |    | ** |    |    |
## |  2 |    |    |    |
## |    | *** |    |    |
## | 43 |    |    |    |
## |    |    | ** |    |
## | 32 |    |    |    |
## |    |    | *** |    |
## | 54 |    |    |    |
## |    |    | *  |    |
## | 66 |    |    |    |
## |    |    |    | *** |
## | 25 |    |    |    |
## |    |    |    |    |
## |  5 |    |    |    |
## |    |    |    |    |
## | 51 |    |    |    |
## |    |    |    |    |
## | 33 |    |    |    |
## |    |    |    |    |
## | 37 |    |    |    |
## |    |    |    |    |
## |  8 |    |    |    |
## |    |    |    |    |
## -----
##
## '***' indicates the most likely position(s) (LOD = 0.0)
##
## '**' indicates very likely positions (LOD > -1.0)

```

```
##
## '*' indicates likely positions (LOD > -2.0)
```

Note that markers 11, 45 and 61 could not be safely mapped to a single position (LOD Score > THRES in absolute value). The output displays the “safe” order and the most likely positions for unmapped markers, where “***” indicates the most likely position and “*” corresponds to other plausible positions.

6. To get the “safe” order, use

```
LG2_f2_safe <- make_seq(LG2_f2_ord, "safe")
```

and to get the order with all markers (*i.e.*, including the ones not mapped to a single position), use:

```
(LG2_f2_all <- make_seq(LG2_f2_ord, "force"))
```

```
##
## Printing map:
##
## Markers          Position
##
## 41 M41           0.00
## 10 M10           8.53
##  2 M2            16.61
## 11 M11           19.26
## 43 M43           22.56
## 32 M32           26.33
## 45 M45           32.28
## 54 M54           34.58
## 66 M66           41.59
## 61 M61           43.26
## 25 M25           48.95
##  5 M5            53.01
## 51 M51           57.36
```



```
## 33 M33                62.99
## 37 M37                67.31
##  8 M8                 73.20
##
## 16 markers            log-likelihood: -997.5821
```

Which places markers 11, 45 and 61 into their most likely positions (between markers 2 and 43, 32 and 54 and between 66 and 25, respectively).

- The `order_seq` function can perform two rounds of the `try_seq` step, first using `THRES` and then `THRES - 1` as threshold. This generally results in safe orders with more markers mapped, but takes longer to run. To do this, type:

```
LG2_f2_ord <- order_seq(input.seq = LG2_f2, n.init = 5,
                        subset.search = "twopt",
                        twopt.alg = "rcd", THRES = 3,
                        draw.try = TRUE, wait = 1,
                        touchdown = TRUE)
```

The output is too big to be included here, so please try to see what happens. In short, for this particular sequence, the `touchdown` step could additionally map one of the three markers (61), but this depends on the dataset. Because there is no other reason to change position of markers 11 and 45 (e.g. biological information), let us use the order with all markers as suggested by the function `order_seq`:

```
(LG2_f2_final <- make_seq(LG2_f2_ord, "force"))

##
## Printing map:
##
## Markers          Position
##
## 41 M41           0.00
## 10 M10           8.53
##  2 M2            16.61
```

```
## 11 M11          19.26
## 43 M43          22.56
## 32 M32          26.33
## 45 M45          32.28
## 54 M54          34.58
## 66 M66          41.59
## 61 M61          43.26
## 25 M25          48.95
##  5 M5           53.01
## 51 M51          57.36
## 33 M33          62.99
## 37 M37          67.31
##  8 M8           73.20
##
## 16 markers      log-likelihood: -997.5821
```

8. Finally, to check for alternative orders, use the `ripple_seq` function:

```
ripple_seq(LG2_f2_final, ws = 5, LOD = 3)

## 41-10-2-11-43-|-32-...
##   Alternative orders:
##    41 10 2 11 43 ... :    0.00
##    41 10 11 2 43 ... :   -0.73
##
## ...-41-|-10-2-11-43-32-|-45-...
##   Alternative orders:
##    10 2 11 43 32 ... :    0.00
##    10 11 2 43 32 ... :   -0.73
##    10 2 11 32 43 ... :   -1.94
##    10 2 32 11 43 ... :   -2.68
##    10 32 2 11 43 ... :   -2.75
##    10 11 2 32 43 ... :   -2.78
##
```

```

## ...-10-|-2-11-43-32-45-|-54-...
## Alternative orders:
## ... 2 11 43 32 45 ... : 0.00
## ... 2 11 43 45 32 ... : -0.34
## ... 11 2 43 32 45 ... : -0.73
## ... 11 2 43 45 32 ... : -1.04
## ... 2 11 32 43 45 ... : -1.94
## ... 2 32 11 43 45 ... : -2.68
## ... 32 2 11 43 45 ... : -2.75
## ... 11 2 32 43 45 ... : -2.78
##
## ...-2-|-11-43-32-45-54-|-66-...
## Alternative orders:
## ... 11 43 32 45 54 ... : 0.00
## ... 11 43 45 32 54 ... : -0.34
## ... 11 32 43 45 54 ... : -1.94
## ... 11 43 32 54 45 ... : -2.68
## ... 32 11 43 45 54 ... : -2.68
##
## ...-11-|-43-32-45-54-66-|-61-...
## Alternative orders:
## ... 43 32 45 54 66 ... : 0.00
## ... 43 45 32 54 66 ... : -0.34
## ... 32 43 45 54 66 ... : -1.94
## ... 43 32 54 45 66 ... : -2.68
##
## ...-43-|-32-45-54-66-61-|-25-...
## Alternative orders:
## ... 32 45 54 66 61 ... : 0.00
## ... 45 32 54 66 61 ... : -0.34
## ... 32 54 45 66 61 ... : -2.68
##
## ...-32-|-45-54-66-61-25-|-5-...

```

```
## Alternative orders:
## ... 45 54 66 61 25 ... : 0.00
## ... 54 45 66 61 25 ... : -2.68
##
## ...-45-|-54-66-61-25-5-|-51-... OK
##
## ...-54-|-66-61-25-5-51-|-33-... OK
##
## ...-66-|-61-25-5-51-33-|-37-... OK
##
## ...-61-|-25-5-51-33-37-|-8-... OK
##
## 25-|-5-51-33-37-8 OK
```

The second argument, `ws = 5`, means that subsets (windows) of five markers will be permuted sequentially ($5!$ orders for each window), to search for other plausible orders. The LOD argument means that only orders with LOD Score smaller than 3 will be printed.

The output shows permuted sequences of five numbers, because `ws = 5`. They can be followed by an OK, if there are no alternative orders with LOD Scores smaller than `LOD = 3` in absolute value, or by a list of alternative orders.

In this example, the first seven windows showed alternative orders with LOD smaller than `LOD = 3`. However, the best order was that obtained with the `order_seq` function (LOD = 0.00). If there were an alternative order more likely than the original, one should check the difference between them and, if necessary, change the order with functions `drop_marker` (see Section 5.8) and `try_seq`, or simply by typing the new order. For that, use `LG2.f2.final$seq.num` to obtain the original order; then make the necessary changes (by copying and pasting) and use the function `map` (see Section 5.8) to reestimate the genetic map for the new order.

9. The `ripple_seq` command showed that the final order obtained is indeed the most likely for this linkage group. The map can then be printed using

```

LG2_f2_final

##
## Printing map:
##
## Markers          Position
##
## 41 M41           0.00
## 10 M10           8.53
##  2 M2            16.61
## 11 M11           19.26
## 43 M43           22.56
## 32 M32           26.33
## 45 M45           32.28
## 54 M54           34.58
## 66 M66           41.59
## 61 M61           43.26
## 25 M25           48.95
##  5 M5            53.01
## 51 M51           57.36
## 33 M33           62.99
## 37 M37           67.31
##  8 M8            73.20
##
## 16 markers          log-likelihood: -997.5821

```

5.6 Genetic mapping of linkage group 1

1. Let us analyze linkage group 1. Extract markers from object `LGs`:

```
LG1_f2 <- make_seq(LGs_f2, 1)
```

2. Construct the linkage map, by automatic usage of the `try` algorithm:

```

LG1_f2_ord <- order_seq(input.seq = LG1_f2, n.init = 5,
                        subset.search = "twopt",
                        twopt.alg = "rcd", THRES = 3,
                        draw.try = TRUE, wait = 1,
                        touchdown = TRUE)

```

The second round of `try_seq` added markers M9 and M44 (try it; results not shown).

3. Get the order with all markers:

```

(LG1_f2_final <- make_seq(LG1_f2_ord, "force"))

##
## Printing map:
##
## Markers          Position
##
## 55 M55           0.00
## 27 M27           7.69
##  9 M9            10.16
##  3 M3            21.27
##  4 M4            23.59
## 42 M42           30.05
## 53 M53           31.59
## 46 M46           35.89
##  1 M1            44.82
## 30 M30           48.87
##  7 M7            55.66
##  6 M6            59.63
## 13 M13           64.45
## 35 M35           68.97
## 58 M58           76.39
## 12 M12           81.95
## 17 M17           85.06
## 31 M31           88.68

```

```
## 34 M34          97.81
## 63 M63          97.81
## 26 M26         106.02
## 40 M40         109.67
## 36 M36         116.65
## 44 M44         118.63
## 29 M29         125.99
## 60 M60         129.36
## 23 M23         137.45
##
## 27 markers      log-likelihood: -1651.548
```

4. Check the final map (results not shown):

```
ripple_seq(LG1_f2_final, ws = 5)
```

No better order was observed (please, try it to see).

5. Print it

```
LG1_f2_final

##
## Printing map:
##
## Markers          Position
##
## 55 M55           0.00
## 27 M27           7.69
## 9 M9            10.16
## 3 M3            21.27
## 4 M4            23.59
## 42 M42          30.05
## 53 M53          31.59
```

```
## 46 M46          35.89
##  1 M1           44.82
## 30 M30          48.87
##  7 M7           55.66
##  6 M6           59.63
## 13 M13          64.45
## 35 M35          68.97
## 58 M58          76.39
## 12 M12          81.95
## 17 M17          85.06
## 31 M31          88.68
## 34 M34          97.81
## 63 M63          97.81
## 26 M26         106.02
## 40 M40         109.67
## 36 M36         116.65
## 44 M44         118.63
## 29 M29         125.99
## 60 M60         129.36
## 23 M23         137.45
##
## 27 markers          log-likelihood: -1651.548
```

5.7 Genetic mapping of linkage group 3

1. Extract markers from object `LGs_f2`:

```
LG3_f2 <- make_seq(LGs_f2, 3)
```

2. Construct the linkage map, by automatic usage of the `try` algorithm and drawing some useful graphics (not shown):


```
LG3_f2_ord <- order_seq(input.seq = LG3_f2, n.init = 5,
                        subset.search = "twopt",
                        twopt.alg = "rcd", THRES = 3,
                        draw.try = TRUE, wait = 1,
                        touchdown = TRUE)
```

We can see that in the second round of `try_seq` marker M56 was added (please, try it). A careful examination of the graphics can be a good source of information about how markers were placed. For more details about how to interpret it, see Section 4.6

3. Now, get the order with all markers:

```
(LG3_f2_final <- make_seq(LG3_f2_ord, "force"))

##
## Printing map:
##
## Markers          Position
##
## 47 M47           0.00
## 19 M19           7.56
## 39 M39           8.94
## 38 M38          15.77
## 49 M49          24.07
## 59 M59          24.49
## 28 M28          31.07
## 14 M14          35.46
## 16 M16          39.99
## 65 M65          45.54
## 62 M62          50.67
## 15 M15          54.67
## 21 M21          58.45
## 24 M24          61.82
## 20 M20          68.26
## 64 M64          68.26
```

```
## 52 M52          73.65
## 48 M48          79.04
## 57 M57          84.99
## 22 M22          88.24
## 18 M18          92.32
## 56 M56         102.04
## 50 M50         104.72
##
## 23 markers      log-likelihood: -1462.52
```

4. Check the final map:

```
ripple_seq(LG3_f2_final, ws = 5)
```

No better alternative order was observed.

5. Print it

```
LG3_f2_final

##
## Printing map:
##
## Markers          Position
##
## 47 M47           0.00
## 19 M19           7.56
## 39 M39           8.94
## 38 M38          15.77
## 49 M49          24.07
## 59 M59          24.49
## 28 M28          31.07
## 14 M14          35.46
## 16 M16          39.99
```

```
## 65 M65          45.54
## 62 M62          50.67
## 15 M15          54.67
## 21 M21          58.45
## 24 M24          61.82
## 20 M20          68.26
## 64 M64          68.26
## 52 M52          73.65
## 48 M48          79.04
## 57 M57          84.99
## 22 M22          88.24
## 18 M18          92.32
## 56 M56         102.04
## 50 M50         104.72
##
## 23 markers          log-likelihood: -1462.52
```

5.8 Map estimation for an arbitrary order

1. If you have some information about the order of the markers, for example, from a previously published paper, you can define a sequence of those markers in a specific order (using the function `make_seq`) and then use the function `map` to estimate the genetic map. For example, for markers M47, M38, M59, M16, M62, M21, M20, M48 and M22, in this order, use:

```
LG3seq_f2 <- make_seq(twopts_f2, c(47, 38, 59, 16, 62, 21, 20, 48, 22))
(LG3seq_f2_map <- map(LG3seq_f2))

##
## Printing map:
##
## Markers          Position
##
## 47 M47           0.00
```

```
## 38 M38          14.49
## 59 M59          23.99
## 16 M16          40.13
## 62 M62          50.60
## 21 M21          57.84
## 20 M20          67.16
## 48 M48          75.76
## 22 M22          84.62
##
## 9 markers          log-likelihood: -947.1229
```

To see the relation between marker names and numbers, use

```
marker_type(LG3seq_f2_map)

## Marker 47 ( M47 ) --> AA : AB : BB (1:2:1)
## Marker 38 ( M38 ) --> AA : AB : BB (1:2:1)
## Marker 59 ( M59 ) --> Not  BB : BB (3:1)
## Marker 16 ( M16 ) --> AA : AB : BB (1:2:1)
## Marker 62 ( M62 ) --> AA : AB : BB (1:2:1)
## Marker 21 ( M21 ) --> AA : AB : BB (1:2:1)
## Marker 20 ( M20 ) --> Not  AA : AA (3:1)
## Marker 48 ( M48 ) --> AA : AB : BB (1:2:1)
## Marker 22 ( M22 ) --> AA : AB : BB (1:2:1)
```

2. If one needs to add or drop markers from a predefined sequence, functions `add_marker` and `drop_marker` can be used. For example, to add markers M18, M56 and M50 at the end of LG3seq_f2_map

```
(LG3seq_f2_map <- add_marker(LG3seq_f2_map, c(18, 56, 50)))

##
## Number of markers: 12
## Markers in the sequence:
```

```
## M47 M38 M59 M16 M62 M21 M20 M48 M22 M18
## M56 M50
##
## Parameters not estimated.
```

Removing markers M59 and M21 from LG3seq_f2_map:

```
(LG3seq_f2_map <- drop_marker(LG3seq_f2_map, c(59, 21)))

##
## Number of markers: 10
## Markers in the sequence:
## M47 M38 M16 M62 M20 M48 M22 M18 M56 M50
##
## Parameters not estimated.
```

5.9 Plotting the recombination fraction matrix

It is possible to plot the recombination fraction matrix and LOD Scores based on a color scale using the function `rf_graph_table`. This matrix can be useful to make some diagnostics about the map.

1. Let us place M38 at the end of linkage group 3 (wrong position):

```
temp_seq <- drop_marker(LG3_f2_final, 38)
```

```
(temp_seq <- add_marker(temp_seq, 38))

##
## Number of markers: 23
## Markers in the sequence:
## M47 M19 M39 M49 M59 M28 M14 M16 M65 M62
## M15 M21 M24 M20 M64 M52 M48 M57 M22 M18
## M56 M50 M38
```

```
##
## Parameters not estimated.

(LG3_f2_wrong <- map(temp_seq))

##
## Printing map:
##
## Markers          Position
##
## 47 M47           0.00
## 19 M19           7.37
## 39 M39           9.16
## 49 M49           23.18
## 59 M59           24.38
## 28 M28           30.43
## 14 M14           34.82
## 16 M16           39.36
## 65 M65           44.90
## 62 M62           50.03
## 15 M15           54.03
## 21 M21           57.82
## 24 M24           61.18
## 20 M20           67.62
## 64 M64           67.62
## 52 M52           73.01
## 48 M48           78.40
## 57 M57           84.36
## 22 M22           87.61
## 18 M18           91.68
## 56 M56           101.38
## 50 M50           103.97
## 38 M38           160.28
##
```

```
## 23 markers log-likelihood: -1589.577
```

Examining the results, we can see there is a big gap at the end of linkage group 3 (between markers M50 and M38, as expected).

2. Let us now plot the recombination fraction matrix:

```
rf_graph_table(LG3_f2_wrong)
```

The recombination fractions are plotted below the diagonal and the LOD Scores are plotted above the diagonal. The color scale varies from red (small distances and big LODs) to dark blue. Clicking on the cell corresponding to two markers, you can see some information about them. For example, clicking on the cell corresponding to markers M47 and M19 you can see their names, types (co-dominant and dominant), recombination fraction ($rf = 0.075$) and LOD Score ($LOD = 23$). Clicking on a cell on the diagonal, some information about the corresponding marker is shown, including percentage of missing data.

We clearly see a different pattern for marker M38. The blue cell, corresponding to markers M50 and M38, indicates a big recombination fraction between these markers as seen before (by clicking, $rf = 0.4049$). Moreover, we can see a group of red cells corresponding to marker M38 and markers M59, M49, M39 and M19. This pattern indicates small recombination fractions between marker M38 and these other markers. Hence, M38 is supposed to be close to them on the map.

3. Because we have enough evidence that marker M38 is misplaced, let us drop this marker and try to map it using the function `try_seq`:

```
temp_seq <- drop_marker(LG3_f2_wrong, 38)
temp_map <- map(temp_seq)
temp_try <- try_seq(temp_map, 38, draw.try = TRUE)

## 38 --> M38 : .....
```

We can see that the most likely position for marker M38 is between markers M39 and M49 (position 4). The patterns on the color matrix are better now. Therefore:

```
(LG3_f2_final <- make_seq(temp_try, 4))

##
## Printing map:
##
## Markers          Position
##
## 47 M47           0.00
## 19 M19           7.73
## 39 M39           7.98
## 38 M38          15.22
## 49 M49          23.60
## 59 M59          23.80
## 28 M28          30.20
## 14 M14          34.66
## 16 M16          39.23
## 65 M65          44.73
## 62 M62          49.89
## 15 M15          53.93
## 21 M21          57.92
## 24 M24          61.10
## 20 M20          67.69
## 64 M64          67.85
## 52 M52          73.08
## 48 M48          78.46
## 57 M57          84.37
## 22 M22          87.67
## 18 M18          91.76
## 56 M56         101.32
## 50 M50         103.96
##
## 23 markers          log-likelihood: -1462.723
```

4. For another example of using function `rf_graph_table`, see Section 4.9.

5.10 Drawing the genetic map

1. We can draw a genetic map for all linkage groups using the function `draw_map`. First we have to create a list of ordered linkage groups:

```
maps_list <- list(LG1_f2_final, LG2_f2_final, LG3_f2_final)
```

Then use it in function `draw_map`:

```
draw_map(maps_list, names = TRUE, grid = TRUE, cex.mrk = 0.7)
```

2. We also can draw a map for a specific linkage group:

```
draw_map(LG1_f2_final, names = TRUE, grid = TRUE, cex.mrk = 0.7)
```

Function `draw_map` draws a very simple graphic representation of the genetic map. However, once the distances are estimated, better map figures can be drawn by the user with any appropriate software. There are several free software that can be used, such as MapChart (Voorrips, 2002).

5.11 Exporting data to R/qtl and QTL Cartographer

Possibly one of the most important applications for a genetic map is its use in QTL mapping studies. In populations such as RILs, F₂ and backcrosses, there are many software options for doing this analysis. Here, we illustrate how to export the genetic map from *OneMap* to the widely used and excellent packages *R/qtl* (Broman et al., 2008) and *QTL Cartographer* (Wang et al., 2010).

1. Using the function `write_map`, let us export the list `maps_list`, defined in the previous section, to a file named "fake_f2_onemap.map":

```
write_map(maps_list, "fake_f2_onemap.map")
```

Notice that the file will be written on the *working directory*, unless specified by the second argument. To set a working directory, see Section 2.5.

2. Now, let us install the *R/ql* package:

```
install.packages("ql")
```

Choose the nearest server location and proceed with the installation. Then, load *R/ql*:

```
library("ql")
```

3. To read the data in *R/ql* we will use the MAPMAKER/EXP format. Two files are needed: the first one is the map file ("fake_f2_onemap.map" in our case); the second one is the raw file written in MAPMAKER/EXP style, which was used in the beginning of this example. This file *must* contain phenotypic information. The simulated data `fake_f2_onemap` contains that information. The location of the raw file can be obtained using:

```
raw_file <- paste(system.file("extdata", package = "onemap"),  
                  "fake_f2_onemap.raw", sep = "/")
```

4. Now we can read the data using the *R/ql* function `read.cross`:

```
fake_f2_ql <- read.cross("mm", file = raw_file, mapfile = "fake_f2_onemap.map")  
  
## --Read the following data:  
## Type of cross:          f2  
## Number of individuals: 200  
## Number of markers:     66  
## Number of phenotypes:  1  
## --Cross type: f2
```

The first argument specifies the format of the data. In our case we used “mm” which stands for MAPMAKER. The second argument (`file`) indicates the raw file in MAPMAKER/EXP format and the third argument `mapfile` indicates the map file produced by *OneMap*

5. Then we can proceed with the analysis. *R/qtl* has several functions to check the map. For example, re-estimating the genetic map within *R/qtl*:

```
newmap <- est.map(fake_f2_qtl, tol = 1e-6, map.function = "kosambi")
```

A comparison of the output of both software can be done with:

```
plot.map(fake_f2_qtl, newmap)
```

For each of the three chromosomes, the left vertical line represents the map estimated by *OneMap* and the right vertical line represents the map estimated by *R/qtl*. The lines linking these two maps indicate marker positions. Thus, we can see that the two maps are almost identical.

6. Finally, we can run an interval mapping analysis for these data using the *R/qtl* function called `scanone` (for details, see *R/qtl* tutorial):

```
fake_f2_qtl <- calc.genoprob(fake_f2_qtl, step = 2)
out_em <- scanone(fake_f2_qtl, method = "em")
out_hk <- scanone(fake_f2_qtl, method = "hk")
plot(out_em, out_hk, col = c("blue", "red"))
```

Here we performed an interval mapping using two methods: mixture models with EM algorithm and Haley-Knott regression. The blue lines indicate the first model and the red lines indicate the latter.

7. We can use *R/qtl* to generate *QTL Cartographer* input files.

```
write.cross(fake_f2_qtl, format = "qtlcart", filestem = "fake_f2_qtl")
```

Again, the file will be written on the *working directory*, unless you specify differently in the `filestem` argument. The files produced this way are ready to be used in *QTL Cartographer*.

6 Final comments

At this point it should be clear that any potential *OneMap* user must have some knowledge about genetic mapping and also the R language, because the analysis is not done with *only one mouse click*. In the future, perhaps a graphical interface will be made available to make this software a lot easier to use.

We do hope that *OneMap* is useful to researchers interested in genetic mapping in outcrossing or inbred-based populations. Any suggestions and critics are welcome.

7 References

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