This guide helps you get started with rSalvador. For more advanced usage, you should consult the accompanying technical manual or use the inbuilt help facility. To get inbuilt help for a function, e.g., newton.LD, type the R command ?newton.LD.

1 Installing rSalvador on the Windows Platform

- Download and install R 3.0.1 or higher version on your computer.
- Make a new folder as your working folder. We recommend naming your working folder c:/rsalvador. Copy the rSalvador distribution file rsalvador_1.2.zip into that new folder.
- Invoke R. rSalvador requires the R package hypergeo, which you can install by the R command

```
install.packages('hypergeo')
```

• rSalvador also requires the R package gdata, which in turn requires the perl software. However, most Windows computers do not have the software perl pre-installed. We recommend Strawberry Perl, which is available on the Internet for free download. Choose the stable version to download and accept all defaults during installation. After installing perl, install the R package gdata with the R command:

```
install.packages('gdata')
```

- Users not interested in the Excel file import capability can skip the installation of both gdata and perl. In this case, you can ignore the harmless warning messages about the unavailability of perl and gdata that you may see each time you load rSalvador.
- Use the R command setwd('c:/rsalvador') to set the new folder as your working directory. If your working folder has a different name, replace 'c:/rsalvador' with the actual name. You can verify your working directory with the R command getwd(). You can check whether the rSalvador distribution file rsalvador_1.2.zip is present in your working folder by executing list.files(). Finally, install rSalvador as follows:

```
install.packages('rsalvador_1.2.zip')
```

2 Installation on the Linux Platform

To begin with, install R and the two R packages (hypergeo, gdata) as described in the above section. Now, follow the following five steps:

1. Make an installation directory and copy the installation file rSalvadorV1.2.tar into the installation directory. For example:

```
mkdir myrsalvador
```

- 2. Untar the installation file that is already in your installation directory (e.g., /myrsalvador): tar xvf rSalvadorV1.2.tar
- 3. A sub-directory rsalvador is now created under the installation directory (e,g., /myrsalvador), but continue to stay in the installation directory. From the installation directory, perform the following checking process (optional but recommonded):

```
R CMD check rsalvador
```

4. If no error messages are generated (except for a few benign warning messages), you are ready to build rSalvador:

```
R CMD build rsalvador
```

5. Finally, install rSalvador by executing

```
R CMD INSTALL rsalvador
```

3 Staring rSalvador

Each time you invoke an R session, you need to load rSalvador with the R command library (rsalvador).

4 Your First rSalvador Calculations

Here we use the Demerec data to demonstrate the basic capabilities of rSalvador. To view this data set, type demerec.data.

• to find the maximum likelihood estimate of m:

```
newton.LD (demerec.data)
```

• to find the 95% confidence interval for m:

```
confint.LD (demerec.data)
```

• To view the iteration details:

```
newton.LD (demerec.data, show.iter=TRUE)
```

• to display the log-likelihood function:

```
plot.likelihood.LD(demerec.data)
```

5 Importing and Exporting Data

There are three ways to transfer data into rSalvador.

• creating a sequence of numbers within R:

```
y=c(0, 16, 20, 2, 2, 56, 3, 361, 9)
```

• importing data from a text file. This data file should have just one column of numbers. It can have one or more memo lines. (Use built-in help for details.)

```
y=import.text.data('example1.txt')
```

• Typically, you may have saved your data in an Excel spreadsheet file, like the accompanying example file example2.xlsx. (See built-in help for details.) To import data from that file, type

```
y=import.excel.data('example2.xlsx')
```

- Now you can repeat the calculations in the above section by replacing demerce.data with the new data variable y.
- Occasionally it may be desirable to save your data (say in y) into a plain text file for future use. This can be done by

```
export.text.data('mytest.txt',y)
```

To read this data file back into rSalvador for analysis:

```
import.text.data('mytest.txt')
```

6 Adjusting for Plating efficiency

We use data from experiment #16 of Luria and Delbruck as an example. This experiment has a plating efficiency of 0.4. You can view the data by typing luria.16.data and learn more by typing ?luria.16.data.

• to find the maximum likelihood estimate of m:

```
newton.LD.plating(luria.16.data, e=0.4)
```

• to find the 95% confidence interval for m:

```
confint.LD.plating(luria.16.data, e=0.4)
```

• To view the iteration details:

```
confint.LD.plating(luria.16.data,e=0.4,show.iter=TRUE)
```

• to view the log-likelihood function graphically:

```
plot.likelihood.LD.plating(luria.16.data, e=0.4)
```

7 Adjusting for Relative Fitness

For the sake of illustration, assume that from a fitness assay the experimentalist learns that the relative fitness is w = 0.21 for the Demerec data. To get a maximum likelihood estimate of m, execute the R command

```
newton.MK (demerec.data, w=0.21) The output is \hat{m}=25.86. To set up a 95% CI for m, execute confint.MK (demerec.data, w=0.21) which yields a 95% CI for m: (22.98, 28.81).
```

8 Comparison of mutation rates

Two mutation rates can be compared by checking whether the two 84% confidence intervals overlap.

```
confint.LD.plating(unlist(crane.data[1]),e=0.1,alpha=0.16)/3.6e9
confint.LD.plating(unlist(crane.data[2]),e=0.1,alpha=0.16)/3.9e9
```

The two confidence intervals for the two mutation rates overlap, and hence the difference is not significant. If terminal cell population sizes are the same in the two experiments, compare.LD performs a likelihood ratio test. In the Newcombe experiments, the difference in N_t between Experiments F and H are small, so a likelihood ratio test is possible.

```
compare.LD(unlist(newcombe.data[6]),unlist(newcombe.data[8]))
```

The likelihood ratio test statistic is $\Lambda = 0.0119$ and the p-value is 0.9130.