# automatic_binner.r[1] manual

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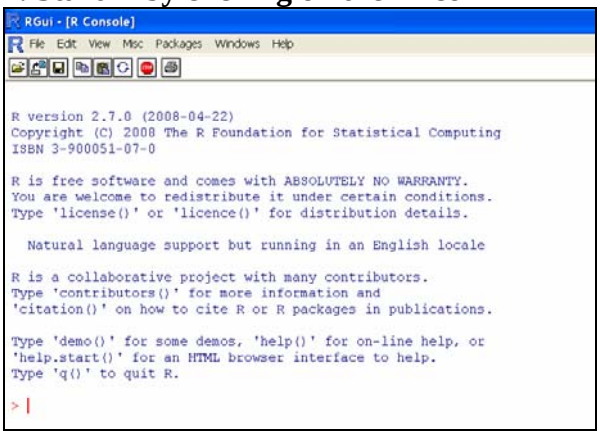
## 1. Preparing the input file

The GeneMapper output file containing the peak sizes, area and height can be copied to your favorite tabulation software.

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Sample | Size | Area | Height |
| 2 | A | 5.05 | 385 | 4248 |
| 3 | A | 6.68 | 708 | 6109 |
| 4 | A | 504.57 | 6369 | 62531 |
| 5 | A | 518.68 | 86 | 2145 |
| 6 | A | 522.45 | 115 | 3180 |
| 7 | A | 525.54 | 120 | 2228 |
| 8 | A | 535.36 | 132 | 3215 |
| 9 | A | 535.97 | 135 | 2477 |
| 10 | A | 537.86 | 717 | 2485 |
| 11 | A | 599.38 | 66 | 1796 |
| 12 | A | 601.69 | 7346 | 107073 |
| 13 | A | 604.69 | 51 | 864 |
| 14 | A | 612.41 | 98 | 2168 |
| 15 | A | 654.33 | 5125 | 65429 |
| 16 | A | 681.66 | 3105 | 32818 |
| 17 | A-1 | 4.74 | 817 | 9001 |
| 18 | A-1 | 5.56 | 752 | 6618 |
| 19 | A-1 | 7.27 | 285 | 2425 |
| 20 | A-1 | 504.46 | 5482 | 58700 |
| 21 | A-1 | 601.63 | 6948 | 100555 |
| 22 | A-1 | 654.37 | 4223 | 57039 |
| 23 | A-1 | 681.55 | 2689 | 25899 |
| 24 | A-2 | 2.7 | 3907 | 31459 |
| 25 | A-2 | 4.33 | 1001 | 12828 |
| 26 | A-2 | 5.72 | 135 | 767 |

Copy the sample, size and area columns **only** to a text file (the height column is not needed). It is important to remove the lines that contain missing information. Column labels must be indicated. An example is given in Data for binner.xls in the "initial" sheet and in the corresponding GeneMapperData1.txt.

## 2. Start R by clicking on the R icon



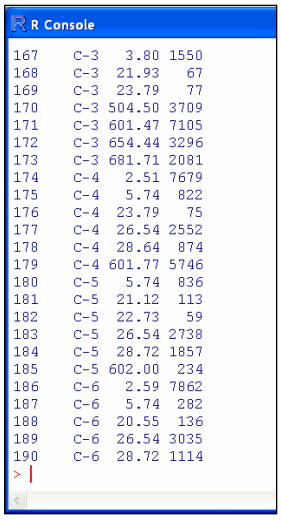

## 3. Load the data into the R workspace

At the prompt (>), indicate in which directory you want to read and write the data (i.e. where you also put your .txt file. The directory should be created beforehand), and press enter. Note that quotes and \\ are used to indicate the path to the directory.

```
>setwd("c:\\R\\ARISA")
```

Then, load the data into the object D by typing the following: (make sure to exactly type the dots, commas, and punctuation signs, as indicated and use " instead of ")

> `D1=read.table("GeneMapperData1.txt",h=TRUE)`



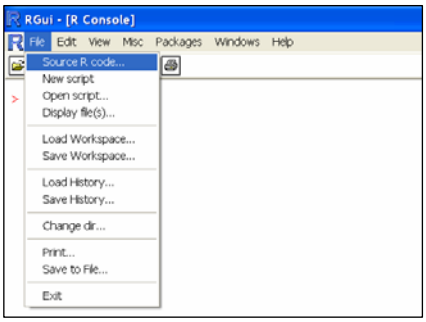

If you now type

>`D1`

You should see your data table appearing in the R console (if it is a big table, you will not see the first rows, but just the end of the table):

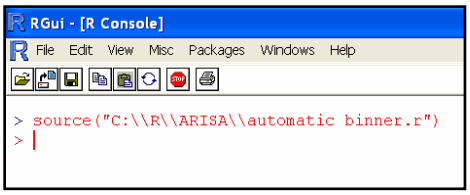
We are now ready to run the R script on the data stored in D.

## 4. Running the automatic binner script



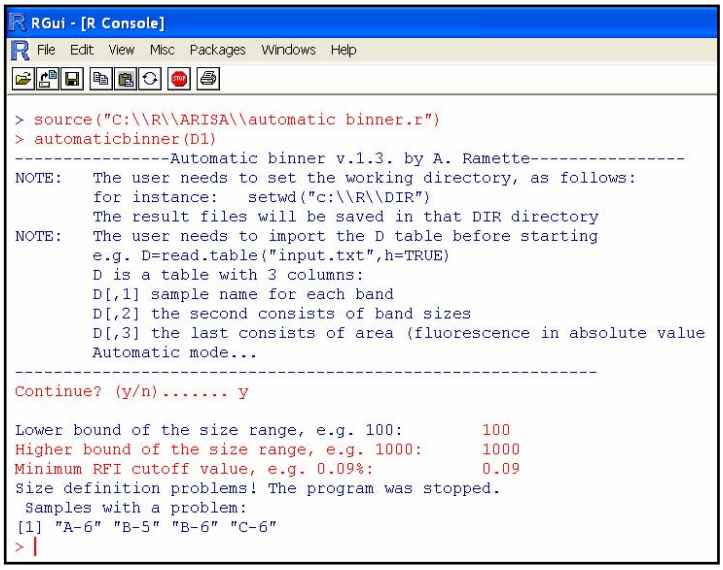

In the menu bar, go to Source R code…

And indicate the location of your saved version of the automatic binner.r script.




>`automaticbinner(D1)`

The script starts by indicating some basic information about the version, expected data format and ask you if you want to proceed. Type "`y`" (without the quotes), if the table D corresponds to the description provided, otherwise type "`n`" and see the points above.



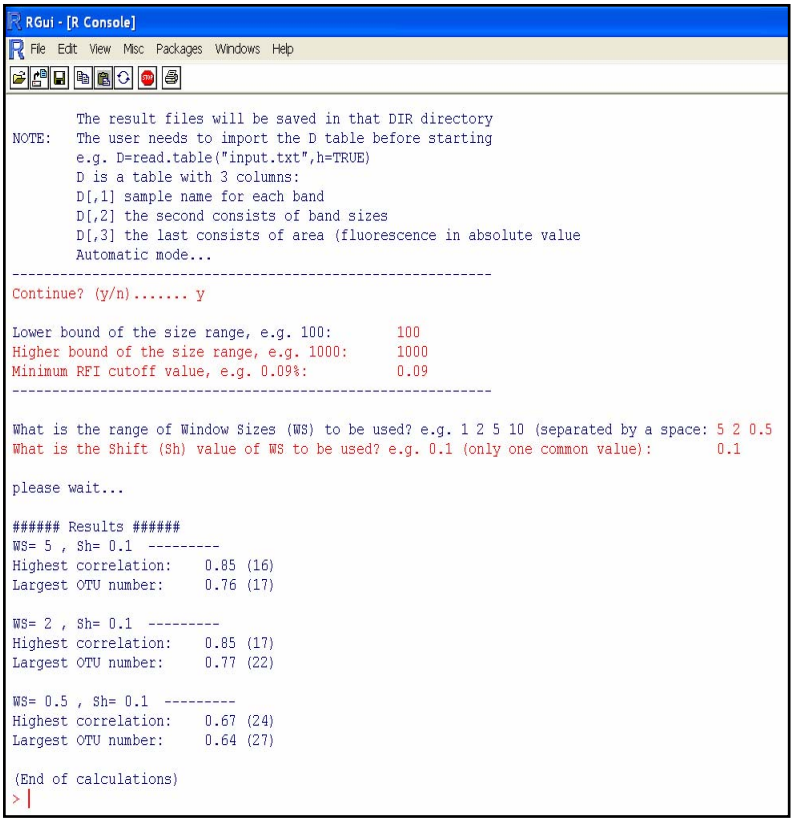

For this example, we can use the following parameters:

- Smallest band size of the range `100`
- Largest band size of the range `1000`
- Minimum RFI cutoff of RFI `0.09`

After few seconds, you should see the following message in the R console:

In this example, the script detected that for some samples, the highest peak size was not fitting in the predefined size range (e.g. for A-6 the largest peak was 28.8 bp while the selected range was 100-1000 bp). Those samples must then be manually removed from the data (i.e. you need to go back to point 1). In this tutorial, the corrected files are found in Data for binner.xls ("corrected" sheet) and the corresponding GeneMapperData2.txt.

Reimport the data to the R workspace:
>`D2=read.table("GeneMapperData2.txt",h=TRUE)`
And run the script again:
>`automaticbinner(D2)`




Again you may try the following parameters:
- Smallest band size of the range `100`
- Largest band size of the range `1000`
- Minimum RFI cutoff of RFI `0.09`
- Window sizes to be screened: `5 2 0.5`
  (i.e. 5, 2, 0.5 bp)
- Shift value (Sh): `0.1`

(only one value is allowed)

You should now obtain the following output:

This time, the script did not stop because no size problems were encountered and the calculations were done.

(Note that the script will also send an error message and stop if the RFI cutoff value is set too high. In the latter case, it would remove too many peaks for calculations to be correctly performed).

**5. Analyzing the results**

The console above indicates, for each window size, the highest correlation values among samples and the respective number of OTUs in parentheses. The first line corresponds to a focus on the highest correlation value among all calculated bin frames, whereas the second line reports the results with a focus on the largest OTU number. This way, each user can decide to use either highest correlation or highest OTU number to choose the corresponding WS value. Typically, a compromise between high correlation value and high number of OTUs has to be found. Note that the program does not return the calculations for the individual bin frames and the interactive_binner.R script should then be used with the chosen WS and Sh values. The console results can be saved by left-mouse selecting the text and copying by right-mouse clicking.

**How to cite the script?**

Ramette, A. (2008) Quantitative molecular community fingerprinting for estimating the abundance of operational taxonomic units in natural microbial communities. *submitted*