

# Instructions for analysing MLPA data when you use GeneScan for peak detection and determination of fragment sizes

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Please note: The automatic diagnosis and quality evaluation produced by the software is only intended to assist in making the final MLPA diagnosis, i.e. we cannot warrant for its usefulness.

## **1 Analysing your own data**

### **1.1 Make one or more copies of the MLPA analysis program**

First of all you should copy the downloaded MLPA analysis program “RH-MLPA-Analysis.mdb” to e.g. “MLPA-Analysis-Original.mdb” and place it beside the “RH-MLPA-Analysis.mdb” program. By doing this it will always be possible to run the demo examples and check the original settings by running “MLPA-Analysis-Original.mdb”.

For routine work we change “RH-MLPA-Analysis.mdb” so that it looks for ABI analysis data supplied on a rewritable CD instead of the demo examples. This is done later and illustrated in figure 2.

### **1.2 Analysing your data exemplified by the P095 probe set**

Proper use of our MLPA analysis program requires that the probe sets be trained with your own data. So assuming that you have trained the system to your data (see 2), and installed the ABI-ExportTabularData program (see 3) we describe how the cytogeneticists at our laboratory do exemplified by the P095 probe set.

**On the ABI 3100 the cytogeneticist:**

- 1) Analyses the samples and prints the electropherograms of the actual run by use of the GeneScan program.
- 2) Exports the analysed sample data to the format needed by the MLPA analysis program by right clicking at the actual run folder and then by clicking ABI-ExportTabularData as shown here:

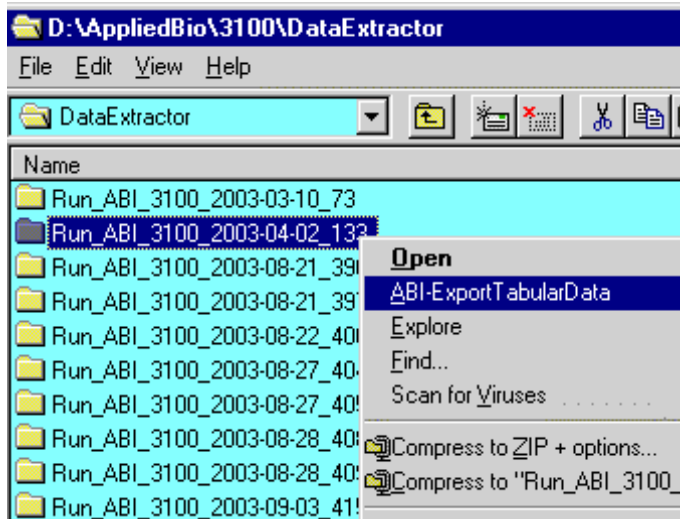


Figure 1. Shows how to export ABI Tabular Data data.

- 3) Sends a copy of the actual run folder to a Rewritable CD by right clicking the run data folder once more, but this time the “Send To” option is clicked and the CD-drive is selected (named “DirectCD Drive (F)” on our ABI 3100 system)

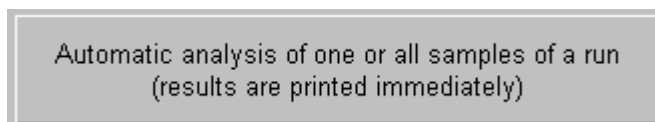
**On another computer having Microsoft Access 2000 installed the cytogeneticist:**

- 1) Starts the program RH-MLPA-Analysis.mdb.

- 2) Select the appropriate MLPA kit:



- 3) Selects the



button.

- 4) Enters a \* in the “MLPA.data file ...” field, and finally clicks at the no. 1 button. Hereafter the program prints a report for each sample. (The actual shown E: drive is a rewritable CD in drive E.)

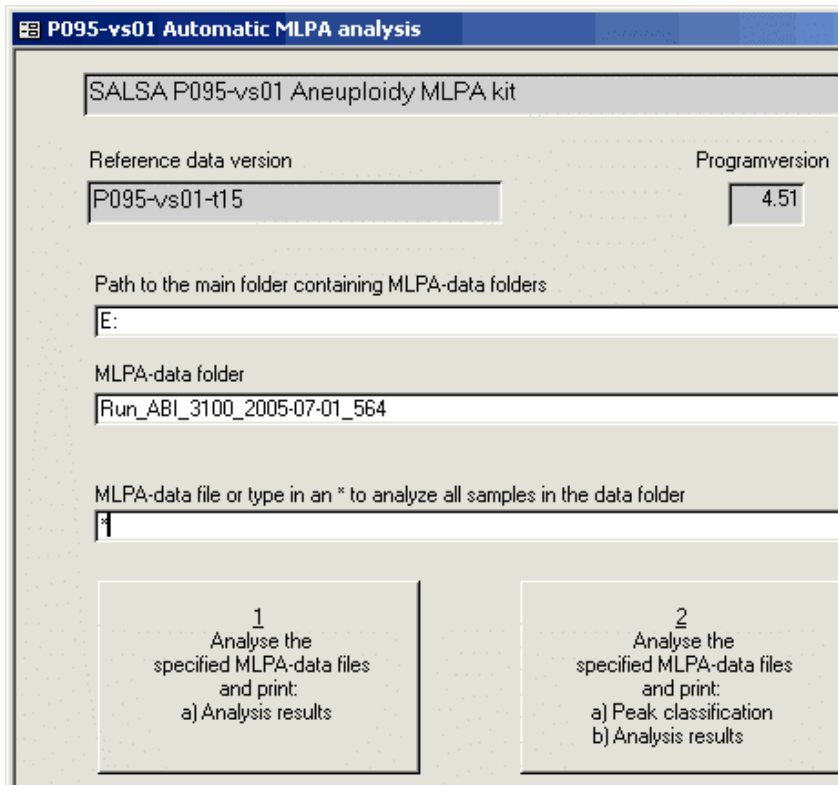


Figure 2. Shows how to analyse your own data supplied on a rewritable CD in drive E.

5) And finally clicks at the no. 1 button, and the program prints a report for each sample.

## 2 Training the analysis program with your own data

Training is now described in a separate documents.

An introduction with an example is given in “instructions-demo.pdf”, and further explanation is shown in “instructions-training.pdf”

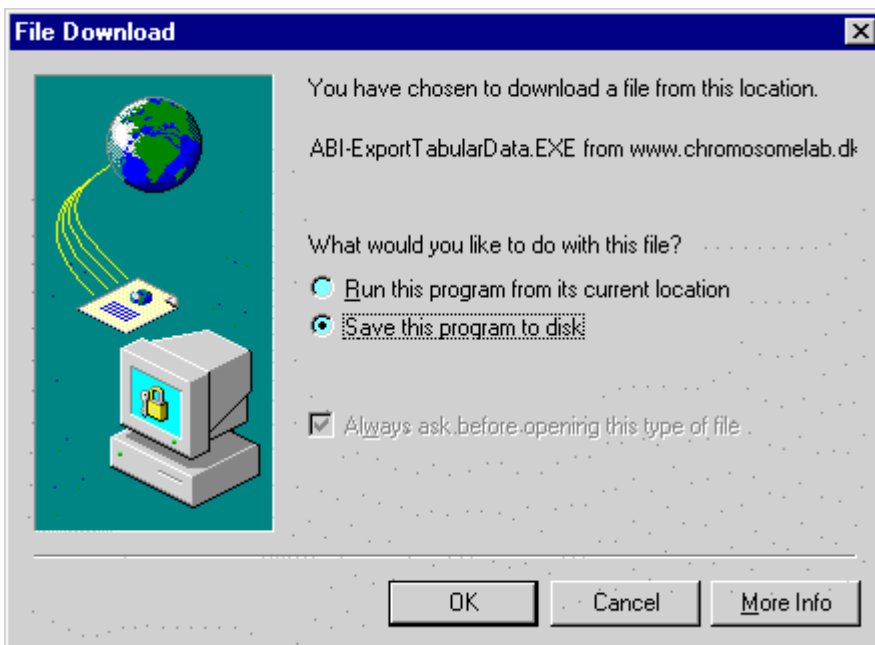
## 3 How to install/uninstall and use ABI-ExportTabularData

Note: The software runs fine on our ABI computer having Windows NT4 and it is tested for both service pack 5 and 6. But we have to say that we cannot give any warranty of any kind, and that it is up to your own responsibility to install and use the program.

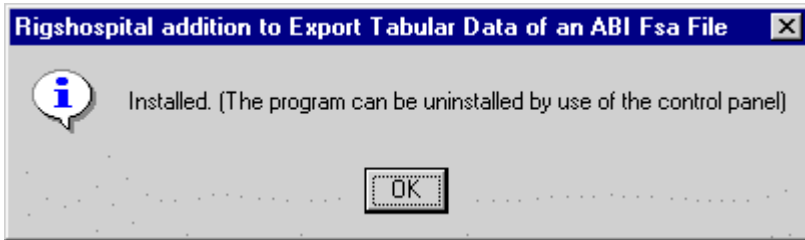
The program is build on basis of the ABI SampleFile Toolkit 3.1 that comes with an ABI 3100 installation, but now it appears that the program needs 3 of the ABI toolkit dll-files to run. These dll files have to be downloaded too and copied into C:\WINNT\system32 (C:\WINDOWS\system32 for Windows XP) before ABI-ExportTabularData can run on your ABI system. See details here: [http://www.chromosomelab.dk/mlpa/download\\_ABI-ExportTabularData.html](http://www.chromosomelab.dk/mlpa/download_ABI-ExportTabularData.html)

### 3.1 Installation

When you at [www.chromosomelab.dk](http://www.chromosomelab.dk) have clicked at “\> Download ABI-ExportTabularData” we propose that you save the installation file on your computer (e.g. the Desktop).

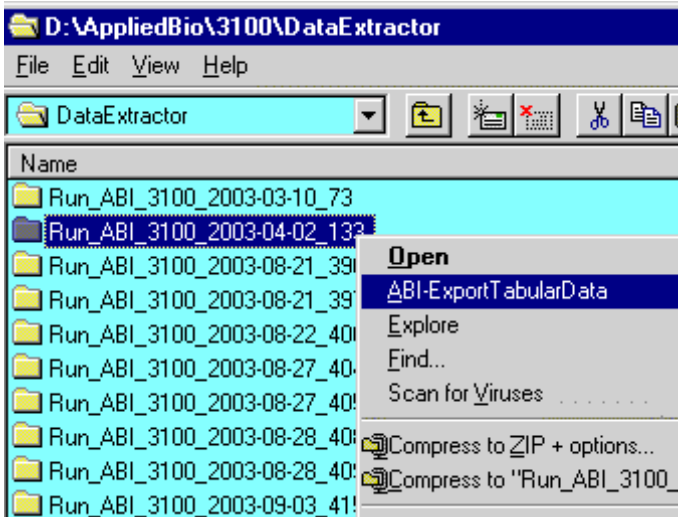


Hereafter you click at the downloaded file (named ABI-ExportTabularData.exe) and answers Yes and Ok to the following two menus:



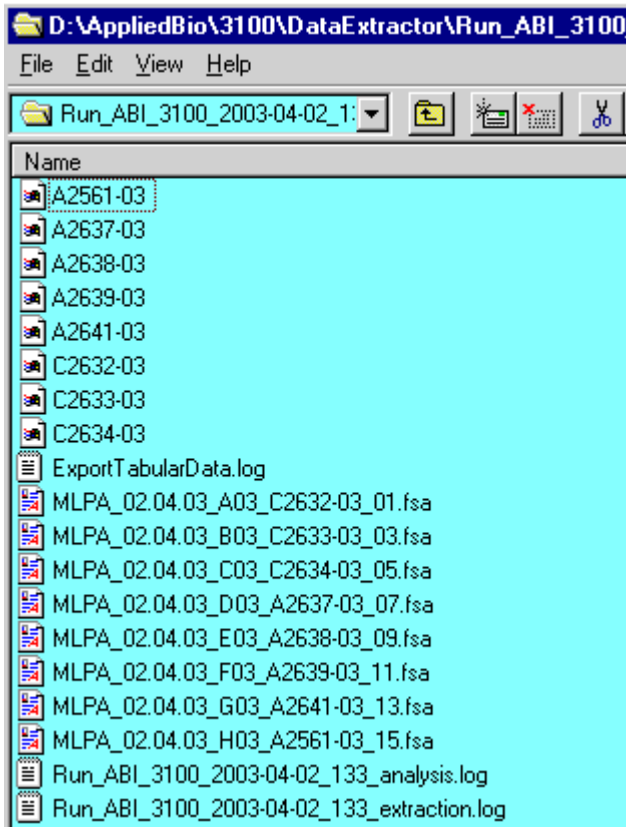
### 3.2 How to use ABI-ExportTabularData to export data produced by the ABI GeneScan program

After having analysed the samples of a run by the GeneScan program on the ABI, you will be able to export the analysed sample data to the format needed by the MLPA analysis program by right clicking at the actual run folder and then by clicking ABI-ExportTabularData as shown here:



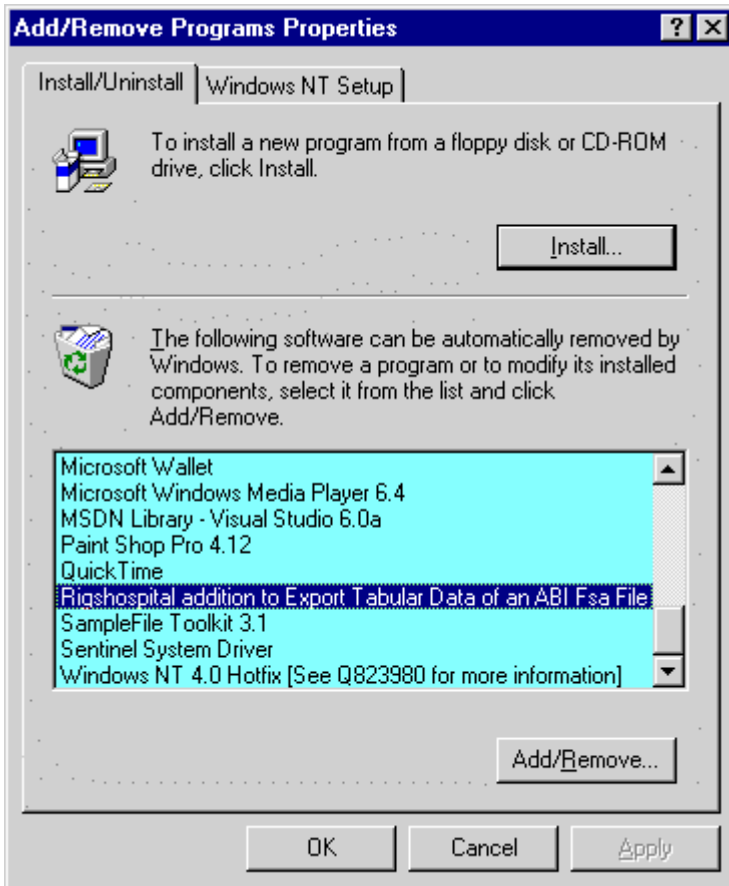
Each exported file is a normal text file without the suffix .txt, and the name is the “Sample Name” that was typed in during the set up of the analysis on the ABI and that is stored internally in the corresponding .fsa ABI file for each sample.

A logfile ExportTabularData.log giving details of each exported file is also generated, but it is very seldom that we look inside the log file. We might look at the log file if not all ABI samples of a run result in a separate file. The log file might reveal that the cytogeneticist e.g. has typed in the same name for more than one sample. An example of an log file is shown in “4 Fileformats”.



### 3.3 Uninstallation

The program can be uninstalled by use of the normal Add/Remove program of the Windows control panel.



## 4 File formats

### 4.1 The logfile of the file export shown in 3.2 looks like this:

ExportTabularData logfile

=====

Working directory: D:\AppliedBio\3100\DataExtractor\Run\_ABI\_3100\_2003-04-02\_133\

SampleName: C2632-03. Capillary: 1.

Collection software version: 1.1 Dye Set: D. Peaks: 66 (B), 12 (G), 6 (Y), 18 (R)

SampleName: C2633-03. Capillary: 3.

Collection software version: 1.1 Dye Set: D. Peaks: 63 (B), 10 (G), 7 (Y), 19 (R)

SampleName: C2634-03. Capillary: 5.

Collection software version: 1.1 Dye Set: D. Peaks: 81 (B), 23 (G), 10 (Y), 22 (R)

SampleName: A2637-03. Capillary: 7.

Collection software version: 1.1 Dye Set: D. Peaks: 66 (B), 35 (G), 6 (Y), 18 (R)

SampleName: A2638-03. Capillary: 9.

Collection software version: 1.1 Dye Set: D. Peaks: 56 (B), 34 (G), 27 (Y), 16 (R)

SampleName: A2639-03. Capillary: 11.

Collection software version: 1.1 Dye Set: D. Peaks: 57 (B), 44 (G), 2 (Y), 16 (R)

SampleName: A2641-03. Capillary: 13.

Collection software version: 1.1 Dye Set: D. Peaks: 56 (B), 50 (G), 2 (Y), 16 (R)

SampleName: A2561-03. Capillary: 15.

Collection software version: 1.1 Dye Set: D. Peaks: 49 (B), 41 (G), 28 (Y), 16 (R)



## 4.2 The format of an exported sample data file

The 69XXY sample file of the P095 examples (C:\RH-MLPA\P095-vs01-examples\69XXY) is used to demonstrate the file format. The files are simple text files without the suffix .txt. There is one line per peak, and each line has 6 fields of data (separated by a tab-key). There is no column header in the files so the explanatory ABI 3100 header text shown below isn't part of the files.

(Note: MLPA analysis doesn't use the columns Minutes and Data Point, so you can place any text in these fields.

MLPA analysis does only need the B-peaks, but if the R-peaks (ROX) are available some statistics are made).

| Dye/Sample Peak | Minutes | Size   | Peak Height | Peak Area | Data Point | This header is not part of the file |
|-----------------|---------|--------|-------------|-----------|------------|-------------------------------------|
| B,1             | 5.94    | 45.31  | 54          | 352       | 2229       |                                     |
| B,2             | 6.17    | 51.31  | 84          | 615       | 2312       |                                     |
| B,3             | 6.21    | 52.30  | 66          | 441       | 2327       |                                     |
| B,4             | 6.33    | 55.29  | 1202        | 26050     | 2373       |                                     |
| B,5             | 6.58    | 61.35  | 326         | 5097      | 2467       |                                     |
| B,6             | 6.81    | 66.96  | 240         | 4640      | 2555       |                                     |
| B,7             | 7.04    | 72.28  | 213         | 4231      | 2639       |                                     |
| B,8             | 7.27    | 78.20  | 352         | 5910      | 2726       |                                     |
| B,9             | 7.66    | 89.16  | 250         | 1617      | 2873       |                                     |
| B,10            | 7.69    | 90.07  | 3722        | 37005     | 2885       |                                     |
| B,11            | 8.82    | 122.26 | 67          | 960       | 3308       |                                     |
| B,12            | 9.18    | 132.93 | 5249        | 54310     | 3443       |                                     |
| B,13            | 9.41    | 140.04 | 4440        | 44210     | 3530       |                                     |
| B,14            | 9.59    | 145.88 | 4554        | 44649     | 3597       |                                     |
| B,15            | 9.80    | 152.67 | 2509        | 24932     | 3675       |                                     |
| B,16            | 9.95    | 157.45 | 141         | 828       | 3731       |                                     |
| B,17            | 9.98    | 158.39 | 1234        | 12215     | 3742       |                                     |
| B,18            | 10.18   | 164.52 | 3822        | 38975     | 3816       |                                     |
| B,19            | 10.38   | 170.83 | 3162        | 32029     | 3893       |                                     |
| B,20            | 10.59   | 177.35 | 3186        | 31887     | 3973       |                                     |
| B,21            | 10.80   | 183.61 | 1780        | 17677     | 4050       |                                     |
| B,22            | 11.06   | 191.62 | 718         | 6917      | 4149       |                                     |
| B,23            | 11.37   | 200.74 | 3372        | 34197     | 4262       |                                     |
| B,24            | 11.66   | 209.78 | 2651        | 27428     | 4372       |                                     |
| B,25            | 11.95   | 218.75 | 3058        | 31438     | 4480       |                                     |
| B,26            | 12.26   | 228.47 | 1948        | 20402     | 4596       |                                     |
| B,27            | 12.55   | 237.95 | 1189        | 12290     | 4708       |                                     |
| B,28            | 12.78   | 245.30 | 2259        | 23606     | 4794       |                                     |
| B,29            | 13.01   | 252.69 | 3291        | 34758     | 4880       |                                     |
| B,30            | 13.31   | 262.41 | 2095        | 22257     | 4992       |                                     |
| B,31            | 13.59   | 271.52 | 1953        | 20647     | 5096       |                                     |
| B,32            | 14.12   | 289.10 | 1603        | 17127     | 5294       |                                     |
| B,33            | 14.41   | 298.82 | 1644        | 18173     | 5402       |                                     |
| B,34            | 14.72   | 310.03 | 1713        | 19036     | 5519       |                                     |
| B,35            | 14.94   | 317.80 | 986         | 11870     | 5601       |                                     |
| B,36            | 15.51   | 337.65 | 1163        | 13700     | 5816       |                                     |
| B,37            | 15.78   | 346.21 | 602         | 6774      | 5917       |                                     |
| B,38            | 16.06   | 355.01 | 1252        | 15785     | 6021       |                                     |
| B,39            | 16.28   | 362.28 | 1042        | 11970     | 6104       |                                     |
| B,40            | 16.84   | 381.27 | 901         | 10613     | 6315       |                                     |
| B,41            | 17.09   | 389.79 | 1203        | 14747     | 6407       |                                     |
| B,42            | 17.33   | 398.28 | 1123        | 13713     | 6497       |                                     |
| B,43            | 17.59   | 407.67 | 682         | 8390      | 6596       |                                     |
| B,44            | 18.06   | 424.58 | 877         | 10905     | 6773       |                                     |
| B,45            | 18.31   | 433.65 | 710         | 9088      | 6867       |                                     |
| B,46            | 18.55   | 442.29 | 874         | 10914     | 6956       |                                     |
| B,47            | 18.79   | 451.12 | 462         | 5938      | 7047       |                                     |
| G,1             | 9.43    | 140.56 | 54          | 203       | 3536       |                                     |
| G,2             | 9.60    | 146.23 | 52          | 244       | 3601       |                                     |
| R,1             | 5.60    | 35.00  | 69          | 630       | 2100       |                                     |
| R,2             | 6.11    | 50.00  | 85          | 769       | 2292       |                                     |
| R,3             | 7.15    | 75.00  | 94          | 790       | 2682       |                                     |
| R,4             | 8.03    | 100.00 | 115         | 891       | 3013       |                                     |
| R,5             | 9.38    | 139.00 | 117         | 772       | 3518       |                                     |
| R,6             | 9.72    | 150.00 | 125         | 1038      | 3644       |                                     |
| R,7             | 10.03   | 160.00 | 128         | 1011      | 3761       |                                     |
| R,8             | 11.34   | 200.00 | 128         | 932       | 4253       |                                     |
| R,9             | 12.82   | 246.41 | 144         | 1193      | 4807       |                                     |
| R,10            | 14.44   | 300.00 | 146         | 1303      | 5415       |                                     |
| R,11            | 15.58   | 340.00 | 147         | 1404      | 5842       |                                     |
| R,12            | 15.90   | 350.00 | 148         | 1374      | 5963       |                                     |
| R,13            | 17.37   | 400.00 | 144         | 1480      | 6515       |                                     |
| R,14            | 18.76   | 450.00 | 149         | 1542      | 7035       |                                     |
| R,15            | 19.86   | 490.00 | 149         | 1619      | 7446       |                                     |
| R,16            | 20.10   | 500.00 | 153         | 1693      | 7539       |                                     |