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 <u>A</u>BI-ExportTabularData as shown here:

🚔 D:\AppliedBio\3100\DataExtractor
<u>F</u> ile <u>E</u> dit <u>V</u> iew <u>H</u> elp
🔁 DataExtractor 💽 🗈 🚈 🔣 🖻 🛙
Name
Bun_ABI_3100_2003-03-10_73
Run_ABI_3100_2003-04-02_132
Bun_ABI_3100_2003-08-21_39
Run_ABI_3100_2003-08-21_39 [*] <u>ABI-ExportTabularData</u>
Bun_ABI_3100_2003-08-22_40
Bun_ABI_3100_2003-08-27_40, Eind
□ Run_ABI_3100_2003-08-27_40! Scan for <u>V</u> iruses
Run_ABI_3100_2003-08-28_40
Bun_ABI_3100_2003-08-28_40
Bun_ABI_3100_2003-09-03_41
Figure 1. Shows how to export ABI Tabular Data data

 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:

Analyse MLPA data on basis of this probeset P095-vs02 Aneuploidy MLPA kit (lot 0505)

button.

3) Selects the

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.)

Automatic analysis of one or all samples of a run (results are printed immediately)

🖴 PO	95-vs01 Automatic MLPA analysis		and the second second
	SALSA P095-vs01 Aneuploidy	MLPA kit	
	Reference data version		
	P095-vs01-t15		4.51
	Path to the main folder containing MLF	PA-data folder	ſS
	E:		
	MLPA-data folder Run_ABI_3100_2005-07-01_564		
	MLPA-data file or type in an * to analyz	ze all samples	in the data folder
			1
	<u>1</u> Analyse the specified MLPA-data files		2 Analyse the specified MLPA-data files
	and print: a) Analysis results		and print: a) Peak classification b) Analysis results

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

3.1 Installation

When you at <u>www.chromosomelab.dk</u> have clicked at "> Download ABI-ExportTabularData" we propose that you save the installation file on your computer (e.g. the Desktop).

File Download	×
File Download	 You have chosen to download a file from this location. ABI-ExportTabularData.EXE from www.chromosomelab.dk What would you like to do with this file? Bun this program from its current location Save this program to diski Always ask before opening this type of file
	OK Cancel <u>M</u> ore Info

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

Rigshospital addition to Export Tabular Data of an ABI Fsa File 🛛 🛛 🔀				
?	Setup a Right-Click Menu Option for exporting the tabularData of an ABI run			
	<u>Yes</u> <u>N</u> o			

Rigshosp	vital addition to Export Tabular Data of an ABI Fsa File 💦 🖡	×
٩	Installed. (The program can be uninstalled by use of the control panel)	I

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 <u>ABI-ExportTabularData</u> as shown here:

😂 D: \AppliedBio\3100\DataE:	ktractor
<u>F</u> ile <u>E</u> dit <u>V</u> iew <u>H</u> elp	
🔄 DataExtractor	- 🗈 🚈 👗 🖻 (
Name	
E Run_ABI_3100_2003-03-10_73	
Run_ABI_3100_2003-04-02_13;	
🚞 Run_ABI_3100_2003-08-21_390	<u>O</u> pen
🚞 Run_ABI_3100_2003-08-21_39	<u>A</u> BI-ExportTabularData
🔁 Run ABI 3100 2003-08-22 40	<u>E</u> xplore
Bun ABI 3100 2003-08-27 40-	<u>F</u> ind
Bun_ABI_3100_2003-08-27_40	Scan for <u>V</u> iruses
🚞 Run_ABI_3100_2003-08-28_40:	Compress to ZIP + options
🚞 Run_ABI_3100_2003-08-28_40:	Compress to "Bun ABI 3100 :
Run_ABI_3100_2003-09-03_41!	

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".

🔄 D:\AppliedBio\3100\DataExtractor\Run_ABI_3100
<u>F</u> ile <u>E</u> dit <u>V</u> iew <u>H</u> elp
🔁 Run_ABI_3100_2003-04-02_1: 💌 💼 🚈 👗
Name
A2561-03
A2637-03
A2638-03
A2639-03
A2641-03
🙍 C2632-03
🙍 C2633-03
C2634-03
ExportTabularData.log
MLPA_02.04.03_A03_C2632-03_01.fsa
MLPA_02.04.03_B03_C2633-03_03.fsa
MLPA_02.04.03_C03_C2634-03_05.fsa
MLPA_02.04.03_D03_A2637-03_07.fsa
MLPA_02.04.03_E03_A2638-03_09.fsa
MLPA_02.04.03_F03_A2639-03_11.fsa
MLPA_02.04.03_G03_A2641-03_13.fsa
MLPA_02.04.03_H03_A2561-03_15.fsa
Bun_ABI_3100_2003-04-02_133_analysis.log
Run_ABI_3100_2003-04-02_133_extraction.log

3.3 Uninstallation

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

Add/Remove	Programs Properties	? ×			
Install/Uninstall Windows NT Setup					
· 😼 🚦	o install a new program from a floppy disk or CD-RON trive, click Install.	1 · .			
	e e e e e e e e e e e e e e e e e e e	<u>ן</u> נ			
	The following software can be automatically removed Windows. To remove a program or to modify its installe components, select it from the list and click Add/Remove.	by ed			
Microsoft \ Microsoft \ MSDN Lib Paint Shop QuickTime	Wallet Windows Media Player 6.4 rary - Visual Studio 6.0a o Pro 4.12 o				
Rigshospital addition to Export Tabular Data of an ABI Fsa File SampleFile Toolkit 3.1 Sentinel System Driver Windows NT 4.0 Hotfix [See Q823980 for more information]					
	Add/ <u>R</u> emove				
	OK Cancel App	y.			

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 not use 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
			8			
B,1	5.94	45.31	54	352	2229	
В,2	6.17	51.31	84	615	2312	
В,3	6.21	52.30	66	441	2327	
B,4	6.33	55.29	1202	26050	2373	
В,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	/8.20	352	5910	2726	
B,9 D 10	7.00	89.10	250	1017	28/3	
Б,10 В 11	7.09	90.07	5722	37003	2003	
D,11 B 12	9.18	132.20	5249	5/310	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,20 D 27	12.20	228.47	1948	20402	4596	
D,27 B 28	12.33	237.93	2250	12290	4708	
D,20 B 20	12.78	245.50	3201	23000	4794	
B.30	13.31	262.41	2095	222.57	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 P 41	10.84	381.27	901	10015	6407	
B,41 B 42	17.09	398.78	11203	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	
к,3 Р 6	9.38 9.72	159.00	11/	112	3318 2644	
к,0 R 7	9.72 10.03	150.00	123	1058	3044	
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	