

Instructions for running the demo examples

The program **RH-MLPA-Analysis.mdb** only requires a computer running Microsoft Access 2000 or 2003 (later Access versions haven't been tested by us).

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After download and unpacking the program can be accessed by clicking at the file RH-MLPA-Analysis.mdb. A simple menu allows you to select one of the MLPA probe sets (P021, P023, P026B, P033, P036B, P036D, P036-E1, P064B, P064-B2, P069, P069-A2, P095, P095-A2, P105-C1, P208-B1, P230-B1, P245, P245-A2, P264-A1, P277-A1, P286-A1, P291-A1) and to analyse your own sample files or the supplied sample files.

For the P023 probe set sufficient normal sample files are supplied to demonstrate how to train the statistics.

For the P026B probe set demonstration files of import of GeneMapper and Peak Scanner produced data are included.

As recommended by Applied Biosystems we have excluded the 250 bp size standard peak during use of GeneScan, GeneMapper and Peak Scanner for all the demonstration samples and for all trained statistics (by a mistake it is not excluded for the P026B probeset). Recently we have also started to exclude the 35 bp standard peak because primer peaks sometime end here and spoils the detection of the real 35 bp standard peak.

Note: The automatic diagnosis and sample quality evaluation made by the software is only intended to assist in making diagnosis, i.e. we cannot warrant for its usefulness, and we make no warranty to the use or performance of the software.

1. Installation of the folder RH-MLPA-v521 on the C-drive

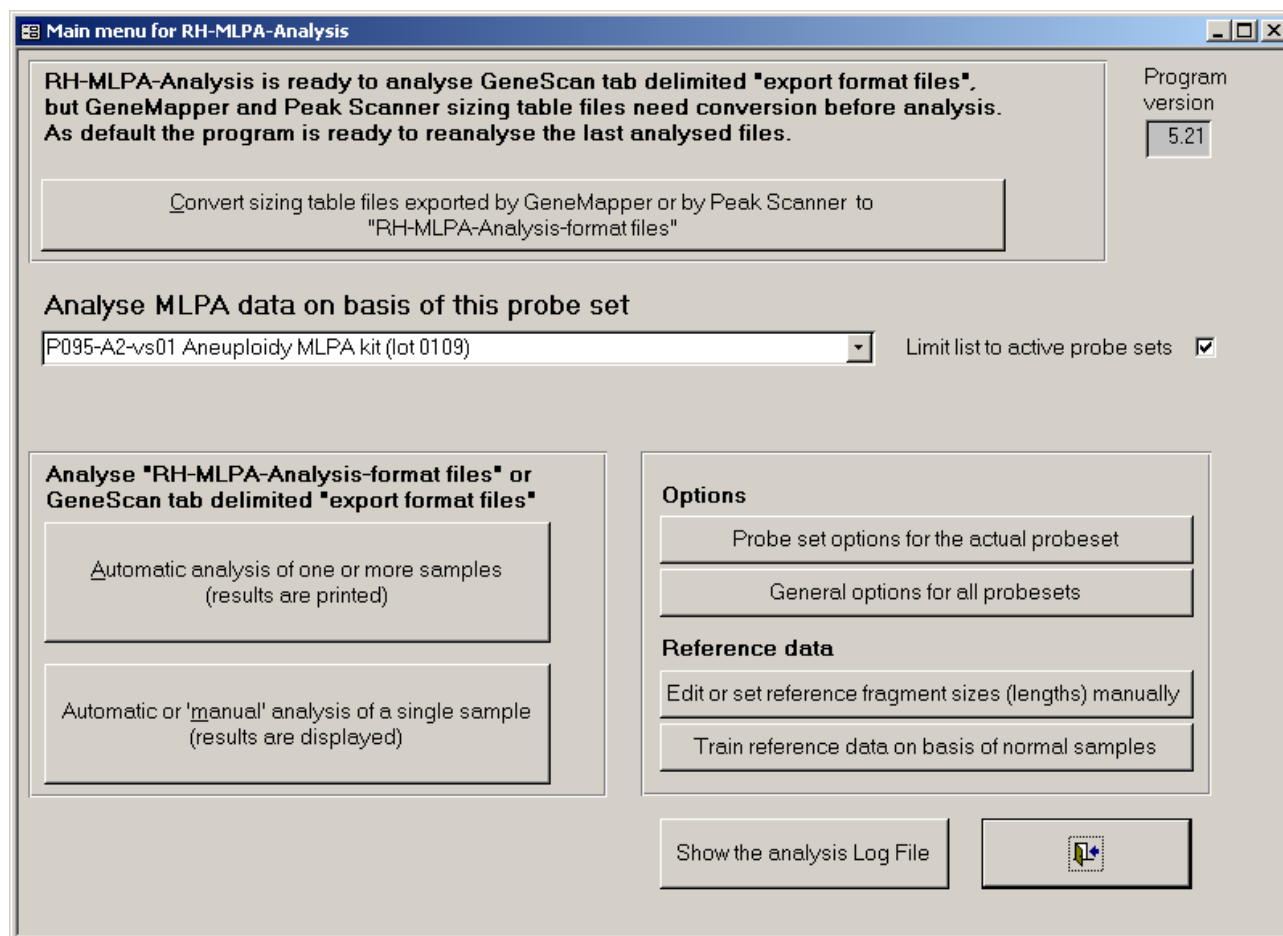
A folder with the program and example data folders for each probe set is packed into "RH-MLPA-v521.zip". After unpacking the folder has to be placed onto the C-drive with the name "RH-MLPA-v521". Otherwise the examples for each probe set cannot be run unless you modify the internal optional settings of folder paths etc. that initially are set in the program to point at the location "C:\RH-MLPA-v521".

For Windows XP the folder RH-MLPA-v521 can easily be installed on the C-drive by clicking at the link [Download the zipped folder RH-MLPA-v521 \(analysis program and examples to analyse\)](#). Hereafter you select Open”, “Extact all files”, click at “Next”, and type in “C:\RH-MLPA-v521” as the selected folder to extract files to. Alternatively you can select to save the zip-file and do the unpacking afterwards.

We included the program version (currently v521) to the folder name so that you can download and test new versions without disturbing older versions that you might use for routine work

2. Run the demo examples

Start Windows Explorer, locate the folder C:\ RH-MLPA-v521, and click at **RH-MLPA-Analysis.mdb**, and this menu appears:



For the first use of RH-MLPA-Analysis it is recommended only to try: “2.1 Selection of a probeset”, “2.2 Automatic analysis and print” and “2.3 Automatic or manually assisted analysis”.

2.1 Selection of a build in MLPA probesets

The selection menu “Analyse MLPA data on basis of this probe set” allows you to select one of the currently build in MLPA probe sets.

This list appears when the list is limited to active probe sets:

P021-vs03 SMA Spinal Muscular Atrophy (lot 0807)
P023-vs02 DiGeorge Syndrome/VCFs MLPA Kit (lot 1005)
P026B-vs01 SOTOS kitt (lot 1005)
P033-vs04 CMT1/HNPP kit (lot 0105, 0605, 0106)
P036-E1-vs02 Human Telomere-3 (lot 1208)
P064-B2-vs01 MR1 MLPA Kit (lot 0208)
P069-A2-vs01 Human Telomere-4 (lot 0808)
P095-A2-vs01 Aneuploidy MLPA kit (lot 0109)
P208-B1-vs01 Human Telomere-6 (2p,3p,6p,8p) (lot 0708)
P230-B1-vs01 Human Telomere-7 (9p,10p,11p,12p) (lot 0609)
P245-A2-vs01 Microdeletion syndromes-1 (lot 0209)
P249-vs01 Human Telomere-8 (17p,18p,19p,20p) (lot 1007)
P264-A1-vs01 Human Telomere-9 (1q,2q,3q,4q) (lot 0408)
P277-A1-vs01 Human Telomere-10 (5q,6q,7q,8q) (lot 0608)
P286-A1-vs01 Human Telomere-11 (9q,10q,11q,12q) (lot 1207)
P291-A1-vs01 Human Telomere-12 (13q,14q,15q,16q) (lot 0508)

By the option settings for each probe set you can decide whether or not a probe set is an active set, and thus limit the displayed list to e.g. a few probesets if you do only use a few of the MLPA sets.

The fragment peak area normalization in the program is able to handle some of the general decrease in probe heights (when looking at the fragment peaks ordered by increasing fragment size), but it cannot handle samples where the slope is extreme large, and it claims that kind of samples to be of too poor quality. The program contains such an example for an earlier and not active version of the P095 probeset (P095-vs2, “Normal-female-example-3_1st_hybridization_Too_high_slope.txt”).

You can access the examples of not active probe sets by removing the mark in

Limit list to active probe sets .

2.2 Automatic analysis and print

The released version of the program is set to be ready to show examples of the probe set “P095-A2”, and it is ready to analyse the sample “P095-A2-trisomy-21.txt”.

2.2.1 Automatic analysis and print of the included (GeneScan-format) examples

Automatic analysis of one or more samples
(results are printed)

The button brings you to a menu from which it is easy to print analysis reports of one (or all samples of a run, useful if all samples of the run have been analysed by the same probe set).

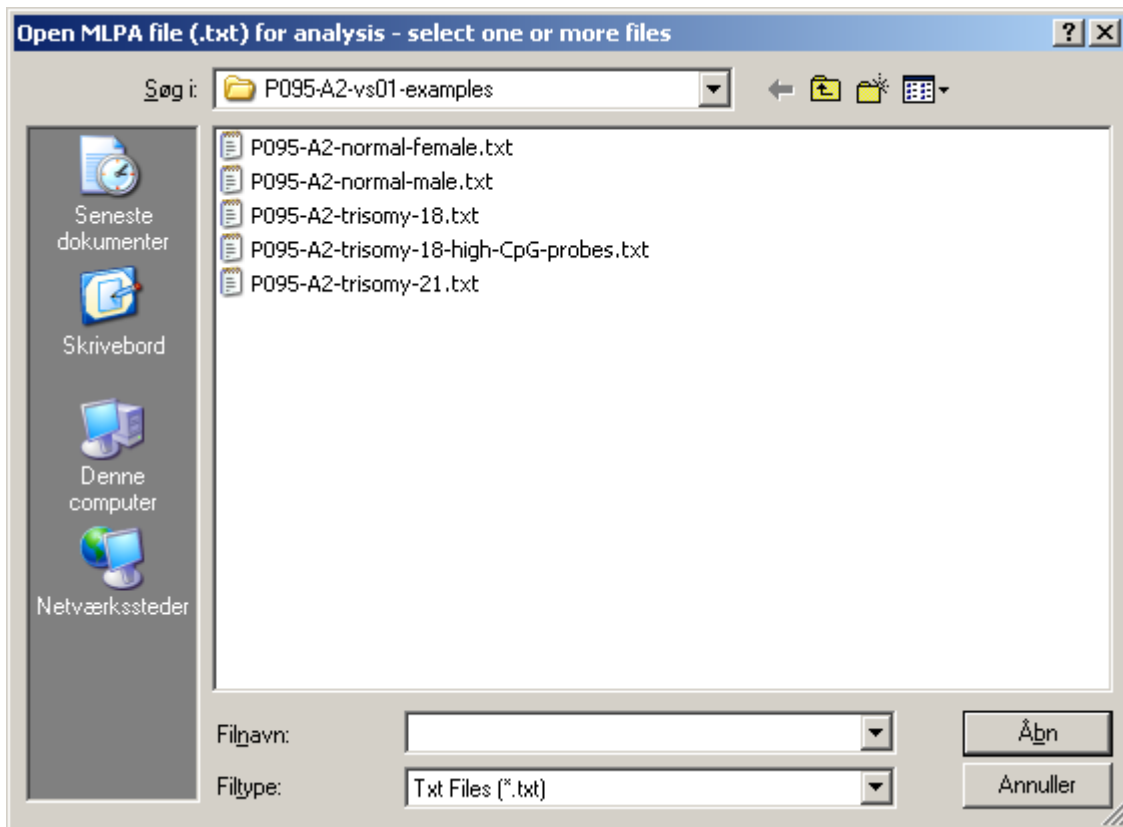
The screenshot shows a software window with the following fields and buttons:

- Program version:** 5.21
- Reference data version:** P095-A2-vs01-t05
- Path to the main folder containing MLPA-data folders:** C:\RH-MLPA-v521
- MLPA-data folder:** P095-A2-vs01-examples
- MLPA-data file (or a note telling about multi selected files):** P095-A2-trisomy-21.txt
(You can also just type in an * to analyze all samples of the data folder)
- Buttons:**
 - 1 Analyse the specified MLPA-data files and print:**
 - a) Analysis results
 - 2 Analyse the specified MLPA-data files and print:**
 - a) Peak classification
 - b) Analysis results
 - Use Windows Dialog to select one or more MLPA files** (with a folder icon)
 - GeneScan tab delimited sizing table export format** (with a document icon)

This menu always remember the last analysed sample, therefore a report should come out on your printer (optimised for A4-sheets) when you click at button 1. Details for each peak found on the ABI will also be printed when button 2 is clicked.

If you type in an * in the field “MLPA-data file ...” before clicking at e.g. button 1 then all samples supplied for the demo are printed.

If you click at the button “Use Windows Dialog to select one or more MLPA files” this Windows Dialog appears (with text in the language of your Access installation):



(If you have an English version of Access this dialog will be on English)

More than one file can be selected by use pressing down the Shift or the Control keys while clicking at the files, or Ctrl+A to select all files after having clicked once inside the file list window.

For samples like P095 where the analysis depends on either female or male DNA and where the analysis program is in doubt whether or not the sample represents female or male DNA, two reports will be printed. One in relation to normal female DNA samples and one in relation to normal male DNA samples.

2.2.2 Automatic analysis and print of the included GeneMapper produced examples

Notes regarding the v521 release:

We ourselves had to give up using the good old GeneScan software because we upgraded to “ABI 3130xl Genetic Analyser” systems that uses GeneMapper. And to streamline the analysis process the program is changed to expect that after GeneMapper file conversion all succeeding MLPA analysis look for files in the folder of the last converted files.

That means that you should close down the program after having tried to convert and analyse the build in GeneMapper or Peak Scanner files of the P026B-vs01 probeset, otherwise the program will assume that the example files of all succeeding probe sets you choose are placed among the converted files.

The actual way of doing analysis is like described for GeneScan-format files (see 2.2.1), but before doing the analysis you have to convert the GeneMapper tab delimited file produced by the GeneMapper program. Such a demonstration file is included for the P026B probe set. (The GeneMapper program generated it after analysis of some P026B samples by selecting the three analysed P026B samples, clicking at “Display Plots”, and when the “Sizing table” is shown then by selecting “File” and “Export Table”, and then to export it as a Tab-delimited text file. This process is described in details in “instructions-analysis-GeneMapper.pdf”).

To convert the GeneMapper demonstration file you

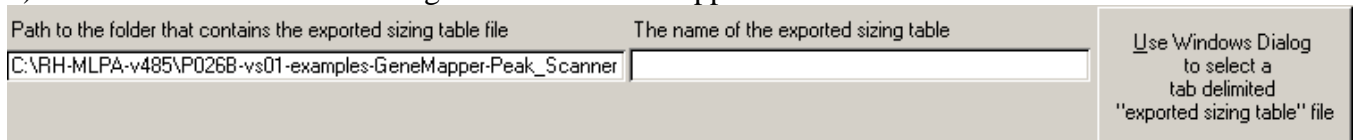
1) First select the P026B probeset:



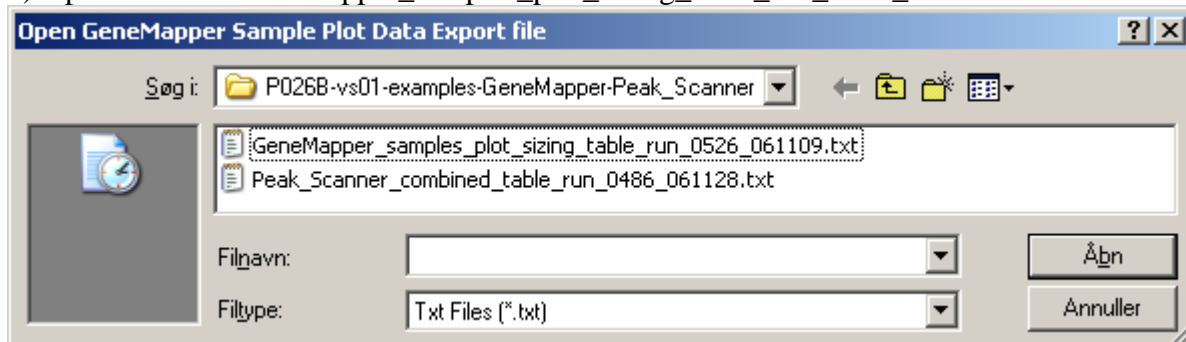
2) Click at the GeneMapper conversion button:



3) Click at “Use Windows Dialog to select a GeneMapper tab ... “:



4) Open the file “GeneMapper_samples_plot_sizing_table_run_0526_061109.txt”:



5) Click at “Click here to convert the selected ‘exported sizing table’ to ‘RH-MLPA-Analysis format’”, and conversion details are displayed during conversion:

Click here to convert the selected "exported sizing table" to "RH-MLPA-Analysis format"

Conversion log

Converted sample data are placed in this folder: '
C:\RH-MLPA-v485\P026B-vs01-examples-GeneMapper-
Peak_Scanner\GeneMapper_samples_plot_sizing_table_run_0526_061109-RH-MLPA'

B-peaks	G-peaks	Y-peaks	R-peaks	Name of the converted sample data file
64	10	1	21	P026B-case_1_E05_2006-11-09.txt
65	21	2	22	P026B-case_2_A05_2006-11-09.txt
62	23	3	21	P026B-case_3_C05_2006-11-09.txt

The GeneMapper file "GeneMapper_samples_plot_sizing_table_run_0526_061109.txt" did thus contain 3 P026B samples, and they are created in a new folder named "GeneMapper_samples_plot_sizing_table_run_0526_061109-RH-MLPA" ready for analysis like described in 2.2.1.

The reason why they are ready to be analysed is that there is a tick mark in "Do it so that the last created sample file becomes the default one for later analysis":

Options are set by general program options

- Remove first 4 chars of converted file names
Useful for removing well position if it is placed first in the sample file name
- Remove last 16 chars of converted file names
Useful for removing date and run no. if these are placed last in the sample filename
- Add .txt to the end of converted files
- Place the converted sample files in the same folder as the sizing table file, otherwise a new folder is made for the sample files
- Do it so that the last used folder becomes the default one for later analysis

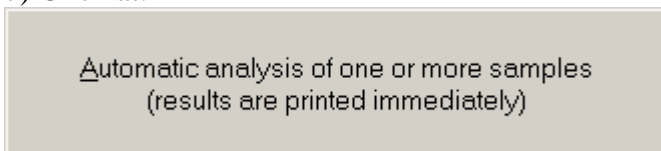
These options can be set by the "General options for all probesets" (see page 2).

Note: For routine use we currently turn on all the options, but the supplied old GeneMapper examples won't work this way as the layout of the *.fsa file names in the supplied examples is different from how we now have set up the system.

6) Click at Exit:



7) Click at:



and you are ready to analyse the GeneMapper data like described in 2.2.1 for GeneScan:

Program version

P026B-vs01 SOTOS kitt (lot 1005) 5.21

Reference data version
P026B-vs01_t00

Select one or more MLPA files by the Windows Dialog button or type in the full path and name to a single MLPA file

Path to the main folder containing MLPA-data folders
C:\RH-MLPA-v511\P026B-vs01-examples-GeneMapper-Peak_Scanner

MLPA-data folder
GeneMapper_samples_plot_sizing_table_run_0526_061109-RH-MLPA

MLPA-data file (or a note telling about multi selected files)
?

(You can also just type in an * to analyze all samples of the data folder)

Use Windows Dialog to select one or more MLPA files

GeneScan tab delimited sizing table export format

1


Analyse the specified MLPA-data files and print:

a) Analysis results

2

Analyse the specified MLPA-data files and print:

a) Peak classification
b) Analysis results



The 7 steps that are needed to import a GeneMapper tab-delimited sample file looks as being many steps to do, but it goes fast when you are used to it, and also when you analyse your own GeneMapper exported samples placed on e.g. a USB-Memory.

2.2.3 Automatic analysis and print of the included Peak Scanner produced examples

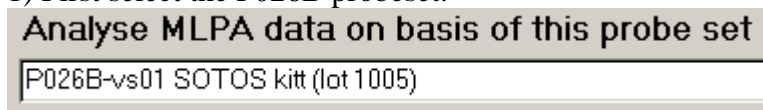
This functionality is almost like to the way GeneMapper samples are analysed (see 2.2.2). Here only a few details regarding the Peak Scanner demonstration file for the P026B probe set are mentioned.

The tab delimited sizing table was generated and exported by the Peak Scanner program after analysis of some P026B samples by: first selecting the two analysed P026B samples (otherwise only one sample is exported), and then by clicking at “Sizing Table View” (otherwise just simple sample information is exported), and then go to the top tool menu bar and select “Export -> Export”. (You are also allowed to select Export -> Export Combined Table”, because this table can also be converted by RH-MLPA-Analysis.)

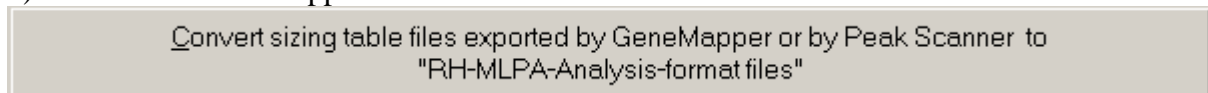
Because Peak Scanner is very much like GeneMapper regarding peak detection we haven’t made a separate instruction document with details like “instructions-analysis-GeneMapper.pdf”.

To convert the Peak Scanner demonstration file you:

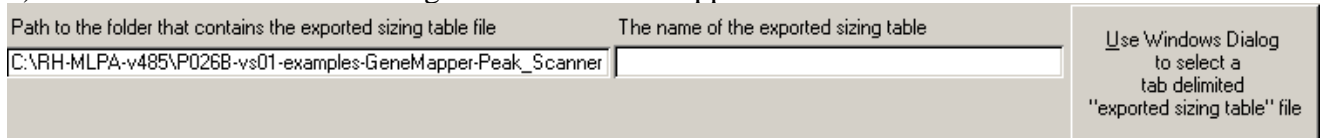
1) First select the P026B probeset:



2) Click at the GeneMapper conversion button:



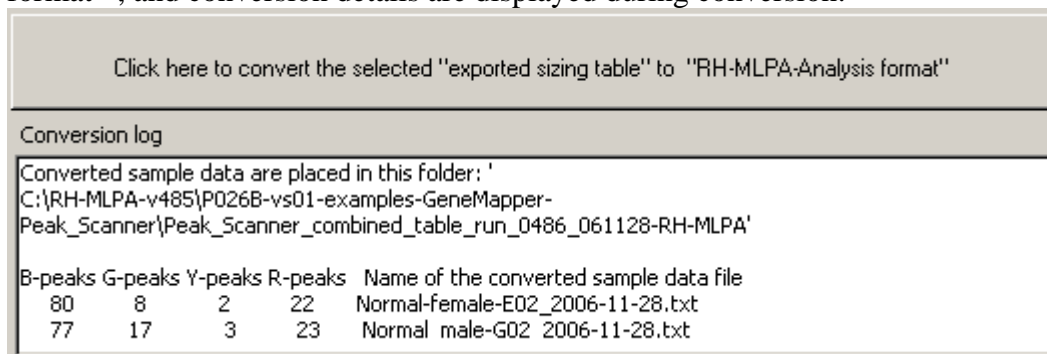
3) Click at “Use Windows Dialog to select a GeneMapper tab ... “:



4) Open the file “Peak_Scanner_combined_table_run_0486_061128.txt”:



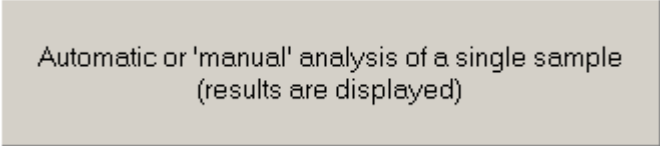
5) Click at “Click here to convert the selected ‘exported sizing table’ to ‘RH-MLPA-Analysis format’”, and conversion details are displayed during conversion:

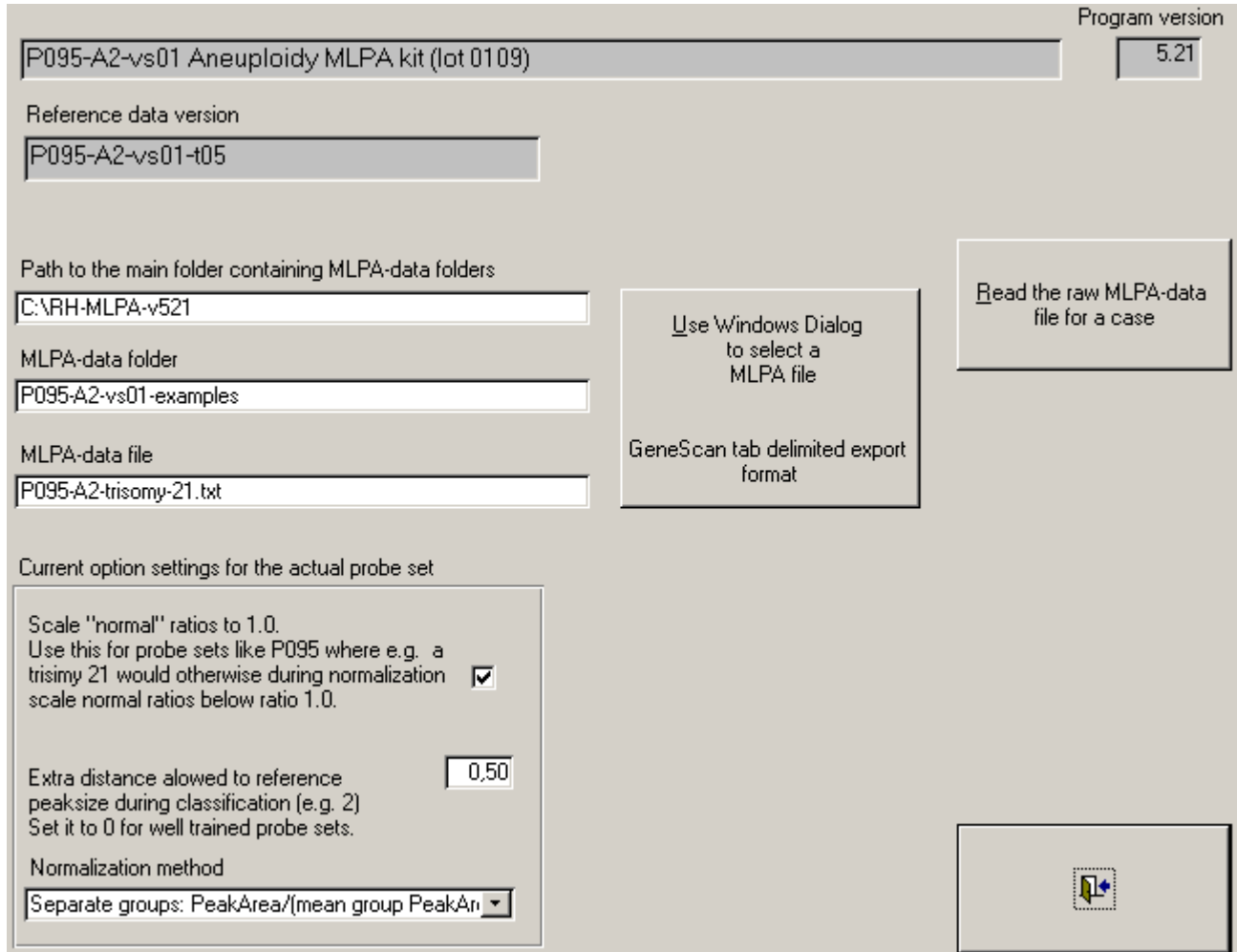


6) Continue like described for GeneMapper in 2.2.2. etc.

2.3 Automatic or manually assisted analysis

Automatic or 'manual' analysis of a single sample
(results are displayed)

The button  brings you to a menu that allows some manual modifications of the analysis before the results are inspected or printed.



Program version 5.21

P095-A2-vs01 Aneuploidy MLPA kit (lot 0109)

Reference data version P095-A2-vs01-t05

Path to the main folder containing MLPA-data folders C:\RH-MLPA-v521

MLPA-data folder P095-A2-vs01-examples

MLPA-data file P095-A2-trisomy-21.txt

Use Windows Dialog to select a MLPA file

GeneScan tab delimited export format


Read the raw MLPA-data file for a case

Current option settings for the actual probe set

Scale "normal" ratios to 1.0.
Use this for probe sets like P095 where e.g. a trisomy 21 would otherwise during normalization scale normal ratios below ratio 1.0.

Extra distance allowed to reference peaksize during classification (e.g. 2)
Set it to 0 for well trained probe sets. 0.50

Normalization method
Separate groups: PeakArea/(mean group PeakArea)



As before, the system remembers the last analysed sample, so try to click the button “Read the raw MLPA-data file for the case”.

If you want to examine the other samples of the demo samples for the actual probe set you just click at the button “Use Windows Dialog to select another MLPA file” to select another MLPA file.

Note: If you change the options then remember hereafter to click at “Read the raw MLPA-data file for the case”, so that the options are applied on the actual sample.

After having read the raw data some extra buttons appear above the exit button:

Program version

P095-A2-vs01 Aneuploidy MLPA kit (lot 0109) 5.21

Reference data version
P095-A2-vs01-t05

Path to the main folder containing MLPA-data folders
C:\RH-MLPA-v521

MLPA-data folder
P095-A2-vs01-examples

MLPA-data file
P095-A2-trisomy-21.txt

Use Windows Dialog to select a MLPA file

GeneScan tab delimited export format

Read the raw MLPA-data file for a case

Check the automatic identification of the fragment peaks

Current option settings for the actual probe set

Scale "normal" ratios to 1.0.
Use this for probe sets like P095 where e.g. a trisomy 21 would otherwise during normalization scale normal ratios below ratio 1.0.


Extra distance allowed to reference peaksize during classification (e.g. 2)
Set it to 0 for well trained probe sets.

Normalization method
Separate groups: PeakArea/(mean group PeakAn

Reference data name
t:XY-ref


Show Peak Classification Report

Show Analysis results Report



- 1) The button “Check the automatic identification ...” is designed to make it possible to e.g. exclude a primer dimer peak that might have ended at the location of a MLPA peak. “Edit label” buttons inside the menu that follows might in seldom cases be useful for reclassifying the peaks. Note: our cytogenetic technologists never use this button for routine analysis.
- 2) The “Reference data name” field is useful for changing the reference data for MLPA probe sets that depend on either female or male DNA. Because sometimes small fragments at the location of the Y-probes for the P095 set might let the automatic procedures assume that the sample originates from male DNA, so in these extreme seldom cases you have to select “t-XX-ref” here.
- 3) Without the option “Scale normal ratios to 1.0 ...” a trisomy 21 of the P095 probe set spoils the normalization so that all peak ratios becomes smaller than expected.
- 4) The option “Extra distance ...” is mostly useful if you get some data from another electrophoresis system and the peaks aren’t placed at the usual locations. For well-trained samples you should be set it to 0.0.
- 5) The two “Show” buttons let you look at the results before printing them. The reports are in MS Snapshot Viewer format. If Snapshot Viewer isn’t installed then Access normally installs it when needed, but we have seen Access installations where no report appears when clicking at the “Show” buttons. There are many solutions to this problem:
 - a. One is to download a Snapshot Viewer from this address: <http://support.microsoft.com/default.aspx?scid=kb;en-us;175274>.
 - b. Another is to double click at the file “47,XY,+21.snp” that is placed next to “RH-MLPA-Analysis.mdb” in the folder” C:\RH-MLPA”. Doing this normally activates the Snapshot Viewer installation program that came with the MS Office package.

2.4 The analysis options and quality limits can be tailored to each probe set

The button  makes it possible for you to tailor both analysis options and the quality limits that are used in reports to indicate whether or not a quality measure is OK.

P095-A2-vs01 Aneuploidy MLPA kit (lot 0109) Program version 5.21

Make this probe set inactive (e.g. if you don't use it)

Analysis option settings for the actual probe set

Scale "normal" ratios to 1.0.
 Use this for probe sets like P095 where e.g. a trisomy 21 would otherwise during normalization scale normal ratios below ratio 1.0. Turn it off for probe sets like P036 that are expected to detect a few abnormal peaks only.

Extra distance allowed to reference peaksize during classification (e.g. 2)
 Set it to 0 for well trained probe sets.

Normalization method
 Separate groups: PeakArea/(mean group PeakArea)



Min. peak height
 Peaks lower than this limit are not detected. Probe sets like P095 does not apply this limit for X and Y probes of male DNA

Quality limits and warnings for the actual probe set.
 On reports the quality becomes "Poor Quality" when passing the "Too low" or "Too high" limit.
 When the "Low" or "High" limits are passed the report only makes a warning to inform you that you are close to the quality limit.

	Too low	Low
Mean A-group area / mean Q-fragment area	<input type="text" value="0,65"/>	<input type="text" value="1,5"/>
Mean CpG-area / mean A-group area	<input type="text" value="0,30"/>	<input type="text" value="0,65"/>
Mean height of 'first half' of probes	<input type="text" value="450"/>	<input type="text" value="800"/>
Mean height of 'second half' of probes	<input type="text" value="280"/>	<input type="text" value="500"/>

	Too high	High
Slope of peak heights "First half"/"Second half"	<input type="text" value="3,00"/>	<input type="text" value="2,50"/>
Mean group CV of weighted ratio	<input type="text" value="0,20"/>	<input type="text" value="0,15"/>
Sum of unidentified peak areas relative to ligated peak areas	<input type="text" value="0,02"/>	
Peaks higher than this limit are marked by an *	<input type="text" value="7000"/>	

Output options

Lot numbers that you have checked or used for training  

Comment to this probeset

This figure illustrates the option settings for the P095-A2-vs01 Aneuploidy MLPA kit (lot 0109) kit. (In Denmark decimals are separated by a "," whereas it might appear as "." on your computer).

For normal routine work you can exclude probe sets that you do not use by this option

Make this probe set inactive (e.g. if you don't use it)

, and set a mark in this field

Limit list to active probe sets

in the main menu to avoid to see the inactive probe sets.

The option "Scale 'normal' ratios to 1.0" is useful for probesets like P095 where all probes are used for normalization and where several probes are expected to appear abnormal in case of aberrant findings. When this option is on the normalization steps are based on median probe areas instead of mean probe areas.

The option "Extra distance ..." is useful if you get some data from another electrophoresis system and the peaks aren't placed at the usual locations. That is the reason why it is set to at least 0.5, to improve the results if you should try to analyse your own data, but often values as large as 3 are needed to make the build in reference data work with sample data generated by another laboratory. For routine work of our well-trained MLPA kits we set this value to 0.

"Normalization method": The peaks can be normalized according to these choices:

- 1) 1 group: $\text{PeakArea}/(\text{mean PeakArea of all peaks})$ (Not advisable)
Each peak area is divided by the mean area of all peaks.
- 2) Separate groups: $\text{PeakArea}/(\text{mean group PeakArea})$
Each peak area is divided by the mean peak area of peaks belonging to the same group of peaks at similar fragment lengths. Currently there are 4 groups used for each of the supplied MLPA probe sets. The normalization groups handle some of the general decrease in probe heights (when looking at the fragment peaks ordered by increasing fragment size), because the groups (A, B, C and D) divide the peaks in groups of increasing fragment size order. See "Instructions-output.pdf" for details of the Group Peak Label letters A, B, C and D that describe the 4 groups for the P095 probe set.

If you intend to change the analysis normalization option then you also have to train the reference statistics by using the same normalization method during training, see "2.7 Training of the reference data on basis of normal samples".

For the **quality options** you can type in separate quality limits for telling that the measured quality figure is "Too low" or "Too high", or just as a warning indicated by "Low" or "High".

The build in settings suit fine to the MLPA samples that we analyse on our ABI 3130xl, i.e. they are not to be regarded as values that suit perfect for all laboratories in the world.

Generally speaking we use the analysis result produced by the RH-Analysis program as long as there are less than 4 quality warnings and there aren't any remarks about too few peaks having a higher peak height than 1000. When having "Poor quality" notes or seeing peaks with unexpected extreme peak ratios (e.g. > 2.5) for a single probe, then we repeat the entire MLPA process using DNA from the same extraction, and then the new analysis often comes out having a good quality. (To help our decision for how to later handle similar cases we collect that kind of results in a ring binder.)

For the P095 MLPA probe set the quality levels are set regarding detection aneuploidy changes for whole chromosomes. That means that a partial deletion of e.g. chromosome 18 sometimes results in quality warnings or note about "poor quality" for the quality measurement "Mean group CV of weighted ratio", because this quality measurement evaluates the overall internal probe variation for peaks that belongs to the same group. For P095 there are one group per chromosome 13, 18, 21, X and Y, and a partial deletion makes it so that the probes for the actual chromosome don't agree on the ploidy of the chromosome. I.e. you can ignore the poor quality warning if you find that it is due to a partial deletion.

By "Mean A-group / mean Q-fragment area" it is measured how high the mean area of the probes that are used for normalization in normalization group A is compared to the mean area of the Q-fragment probes. When this ratio is small then either ligation has failed or the amount of sample DNA is insufficient.

For probe sets that include D-fragment control peaks these peaks are used to evaluate denaturation and hybridization. The program compares

By "Mean CpG-area / mean A-group area" it is measured how high the mean area of the two CpG probes is compared to the mean area of the probes that are used for normalization in normalization group A. When this ratio is small then the denaturation of the sample DNA may have been incomplete. Currently we don't have enough statistics to set these limits, so they are set low except for the P245 probeset (see the bottom of this page <http://www.chromosomelab.dk/mlpa/examples-P245-vs2.html>).

(The program currently also checks whether the D-fragment probe 2p14 likewise is low as this might indicate an incomplete hybridization. In case of a too low value this text will be added to the result sheet "The small 2q14 area indicates poor hybridization!".)

	Too low	Low
Mean A-group area / mean Q-fragment area	0,65	1,5
Mean CpG-area / mean A-group area	0,30	0,65

Actual settings for the P095-A2-vs01 probeset

By "Mean height of 'first half' of probes" it is checked whether or not the mean area of the probes that are used for normalization in normalization group A and B is high enough.

By "Mean height of 'last half' of probes" it is checked whether or not the the mean area of the probes that are used for normalization in normalization group C and D is high enough.

By "Slope of peak heights 'First half'/'Last half'" it is checked whether or not the general decrease in probe heights is too large when ordered by increasing fragment size. Computed as mean height of the probes in the groups A and B divided by the mean height of the probes in the groups C and D.

For probe sets that have a large group of control probes the quality measure "Mean. group CV of weighted ratio" is replaced by "CV of Control Probes" (e.g. P021).

	Too high	High
Slope of peak heights "First half"/"Second half"	3,00	2,50
CV of Control Probes	0,20	0,15

This is illustrated here:

It is possible to save probe ratios of each probe for each analysed sample in a tabulator separated text file (currently only for the P021 probeset).

Output options

Save results to P021-vs03_results.txt

A further explanation of some of the different quality measures is available here:

- "Instructions-output.pdf" that is available here <http://www.chromosomelab.dk/mlpa/download.html> as a "Detailed description of the output sheets exemplified by the P095 kit (pdf)"
- "Automatic analysis of multiplex ligation-dependent probe amplification products (exemplified by a commercial kit for prenatal aneuploidy detection). Gerdes T, Kirchhoff M, Bryndorf T., Electrophoresis. 2005, 26, 4327-4332"

Finally you can update the lot number and a comment line. The lot number you specify (e.g. after having trained the statistics to your own samples) will be printed on all reports.

2.5 General options for all probesets

Currently there are these few options that are common to all probesets.

General options	
Show only ".txt" files when asked to select MLPA files for analysis. Useful if the sizing table files all have names ending on ".txt".	<input checked="" type="checkbox"/>
Options for converting GeneMapper or Peak Scanner sizing table files to "RH-MLPA-Analysis-format files"	
Remove first 4 characters of converted file names. Useful for removing well position if it is placed first in the sample filename.	<input type="checkbox"/>
Remove last 16 characters of converted file names. Useful for removing date and run no. if these are placed last in the sample filename.	<input type="checkbox"/>
Add ".txt" to the end of the name of converted sizing table files.	<input checked="" type="checkbox"/>
Place the converted sample files in the same folder as the sizing table file, otherwise they are placed in a new folder having same name as the sizing table but with "-RH-MLPA" added to the end.	<input type="checkbox"/>
Do it so that the folder with the last converted sample files becomes the default one for subsequent analysis, but only until you restart the program.	<input checked="" type="checkbox"/>

2.6 Simple manual adjustment of reference fragment sizes (lengths)

Edit or set reference fragment sizes (lengths) manually

This button should not be used when looking at the demonstration samples.

The button and submenu are useful for analysing data that come from another system than an ABI 3100, e.g. a MegaBACE system. We have been successful in analysing some MegaBACE data on basis of the normal references based on ABI 3100 measurements, by just modifying the reference data locations of the probe peaks so that they become close to the peak locations of the MegaBACE system.

This button and submenu are also useful for doing the first training of a new MLPA probe set as the training procedures need some good estimates of the actual peak location for each peak before it is possible to generate proper statistics and new reference data on basis of a set of normal samples.


2.7 Training of the reference data on basis of normal samples

Train reference data on basis of normal samples

If you 1) select the **P023-vs01** probe set

(currently not active so remove the mark in **Limit list to active probe sets**)

and 2) click the “Train reference data on basis of normal samples” button then the necessary files for doing a simple training are supplied in the demo cases.

Current MLPA probe set	Current MLPA-training-list file name	No. of samples used for training	Program version
P023-vs01	P023-vs01-t02.txt	39	5.21
Path to the main folder containing MLPA-data folders		Last analysed sample (updated currently during training etc.)	
C:\RH-MLPA-v521\P023-vs01-Training\Data		MLPA-data folder: _2005-06-16_509 P023	
Path to the main folder containing MLPA-traininglist folders		MLPA-data file: P023-N39	
C:\RH-MLPA-v521\P023-vs01-Training		Number of Y chromosomes: <input type="text"/>	
Folder that contains the MLPA-traininglists		Reference data name: t-ref	
Norm-lists			
New MLPA-training-list file name			
<input type="text"/>			
No. of samples			
<input type="text"/>			
Current option settings for the actual probe set			
Scale "normal" ratios to 1.0. Use this for probe sets like P095 where e.g. a trisomy 21 would otherwise during normalization scale normal ratios below ratio 1.0. (Only used during analysis, i.e. not during training.) <input checked="" type="checkbox"/>			
Extra distance allowed to reference peaksize during classification (e.g. 2) Set it to 0 for well trained probe sets. <input type="text" value="0.50"/>			
Normalization method Separate groups: PeakArea/(mean group PeakArea) <input type="text"/>			
Analyse the samples listed in the MLPA-training-list file. Print Report and update the logfile statistics for each sample.		Train the program by the samples listed in the MLPA-training-list file. Can only do full training, i.e. not add a few new cases to the previous training!	
Analyse the samples listed in the MLPA-training-list file. But do only update the Logfile statistics for each sample.			

Training on basis of the supplied 39 normal samples for probe set P023 will take place when you:

- 1) Type in the text “P023-vs01-t02.txt” in the empty field “New MLPA-training-list file name” or copy it from the field “Current MLPA-training-list file name”.
- 2) Hereafter click at the button “Train the program by ...”.

The “MLPA-training-list file” has to contain the name of each normal sample file that is going to contribute to the training. E.g.:

```
_2005-05-12_376\P23-N1
_2005-05-12_376\P23-N10
_2005-05-12_376\P23-N11
_2005-05-12_376\P23-N12
_2005-05-12_376\P23-N13
_2005-05-12_376\P23-N14
```

See further details in “2 Training the analysis program with your own data” in the document “instructions-training.pdf”.

If you click at “... Print Report and update ...” then no training takes place. Instead 39 report sheets are printed on basis of the current trained statistics for the P023 probeset.

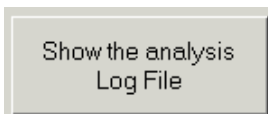
(This is a good method to check whether or not normal samples listed in the “MLPA-training-list file” are rated as having good quality when compared to the current reference data statistics. The button isn’t restricted to handle normal cases only. You could e.g. create a “MLPA-training-list file” that only has the names of abnormal cases you have detected. And then uses this button to print e.g. all your abnormal cases before or after training based on a new set of normal cases.)

The purpose of the button “... Do only update the Logfile ...” is the same as for the previous button, but this time only an internal logfile is updated by one results summary record per sample. The logfile was designed to assist for our publications regarding the P001 and the P095 MLPA probe sets, so it might not contain all essential summary data for the other type of probe sets. The logfile “t-Case-Log” and all trained reference data statistics files can be inspected (see later in paragraph 3 and 4).

The option “Scale normal ratios to 1.0 ...” is only used for the “Print Report ...” and “Do only update Logfile ...” buttons, i.e. the scaling is not applied while the statistics is being trained.

The option “Extra distance ...” might be useful for the very first training, because the actual peak locations represented by the current reference set might be too long away for the system to recognize the peaks, when no extra distance is added.

3. See the most essential fields of the log file





By clicking at the button in the main menu of the program the most essential fields of the logfile are displayed for each analysed MLPA sample. (In paragraph 4 it is described how to see all fields of the logfile).

The most essential fields of the analysis log file RH-MLPA-Analysis program version: 4.53


ID	Date	Raw Data Folder	Raw Data Name	Training Specification	Ref. Data Name	Final Score Note	Max Dist In SD	Quality Note
6	31-10-2005	P095-vs01-examples	Trisomy-13	P095-vs01-t15.txt	t:XY-ref	Trisomy 13	6.5 13q14.2	
7	31-10-2005	P095-vs01-examples	Trisomy-18 {maybe-X-pol}	P095-vs01-t15.txt	t:XX-ref	Trisomy 18	10.3 18q21.1	
8	31-10-2005	P095-vs01-examples	Trisomy-21-LowAmountD	P095-vs01-t15.txt	t:XY-ref	Trisomy 21	14.5 21q22.11	1 quality warning!
9	31-10-2005	P095-vs01-examples	47>XY,+21	P095-vs01-t15.txt	t:XY-ref	Trisomy 21	9.9 21q22.13	
10	31-10-2005	P095-vs01-examples	Del4pEnh18q	P095-vs01-t15.txt	t:XX-ref	Increased ratio of chromosome 18	8.5 18q21.1	
11	31-10-2005	P095-vs01-examples	63>>>Y	P095-vs01-t15.txt	t:XY-ref	Contamination by mat. DNA or 69>>>XY?	5.4 18q21.33	1 quality warning!
12	31-10-2005	P095-vs02-examples	Normal-female	P095-vs02-t01.txt	t:XX-ref	Normal 13, 18, 21, XX		
13	31-10-2005	P095-vs02-examples	Normal-male	P095-vs02-t01.txt	t:XY-ref	Normal 13, 18, 21, XY		
14	31-10-2005	P095-vs02-examples	Trisomy-21	P095-vs02-t01.txt	t:XY-ref	Trisomy 21	10.9 21q11.2	

Use PageUP, PageDown, Ctrl+Home and Ctrl+End to scroll up and down the list.

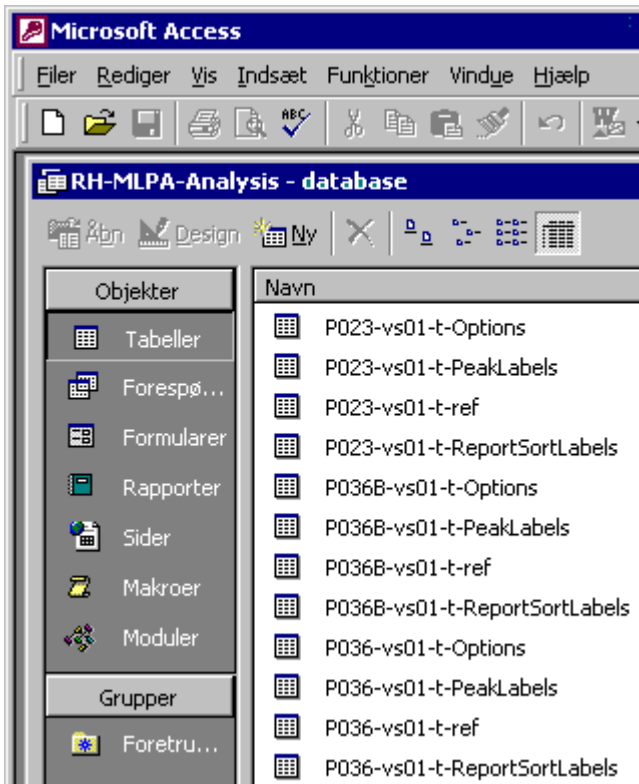
By clicking at the left margin record selectors the Arrow-Up/Down keys can also be used to scroll the list, and individual records can be deleted by pressing the delete-key.

Delete all records



4. How to look at reference data and source codes that are build into the program

All reference data files, the logfile “t-Case-Log”, and program source codes can be inspected by clicking at the top right “close button” of the very first probe set selection menu .

Then something like this appears (the language depends on the Access-version):



The logfile “t-Case-Log” is placed near the bottom of the list of tables. Another possibility for looking at the log file data is to click at the “Queries” (“Forespørgsler” in Danish) button above and then select one of the queries “q-ShowTheLogFile”, “q-ShowTheLogFileFemaleData” or “q-ShowTheLogFileMaleData”.

You are welcome to change the behaviour of the program, e.g. if you only use one MLPA probe set you could set up the start page to e.g. always start with the form like shown in “2.2 Automatic analysis and print”.

If you modify the code in a way that might be useful for other users then send me (tommy.gerdes@rh.regionh.dk) a copy of the changes and I might incorporate them into the distributed program. Especially bug fixes would be appreciated.

You are also welcome to send an e-mail to inform me whether or not you can use the program, and whether or not you like to get an e-mail when a new version of the program is ready for download. You are not allowed to sell the program.