

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

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.

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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”.

When using your routine version of “RH-MLPA-Analysis.mdb” it will always separately for each probe set remember the location of the latest analysed sample and the latest used option settings. E.g. for routine work with GeneMapper we export the MLPA fragment peak data (sizing tables) to a USB-Memory, and after analysis of such data “RH-MLPA-Analysis.mdb” remembers the new location of data and forgets the location of the build in examples for the actual probe set.

This document is mostly written to show how we handle routine data analysis of MLPA kits, whereas the document “instructions-demo.pdf“ contains the general description of how to change options, how to manually change the automatically determined peak sizes of a sample, and how to apply female reference data if the system automatically applied male reference data (for probe sets like P095).

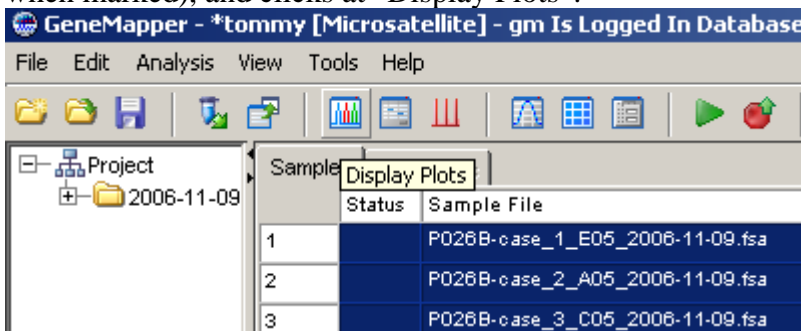
It has to be said that RH-MLPA-Analysis is most efficient if you export several samples at the same time (e.g. all samples of the same MLPA probe set of a run) to one single file, because in RH-MLPA-Analysis you convert a GeneMapper file to a new folder whereby all the individual samples of the GeneMapper file are placed as separate files. These separate files are then ready to be analysed by RH-MLPA-Analysis in the first designed way as if the files were generated and exported sample by sample by the GeneScan program that earlier came with ABI systems.

1.2 Analysing your data exemplified by the P026B probe set

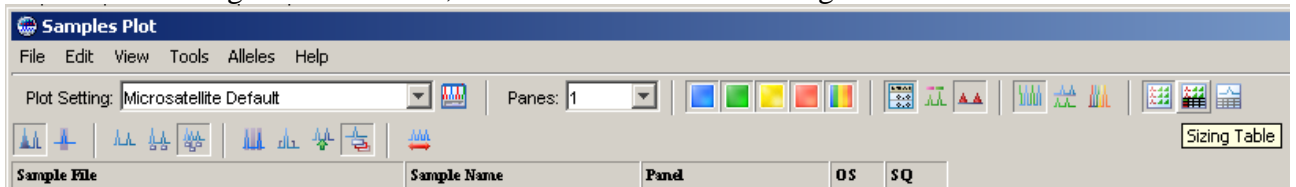
Proper use of our MLPA analysis program requires that the probe sets are trained with your own data. So assuming you have trained the system to your data (see “2 Training the analysis program with your own data”) we describe how the cytogeneticists at our laboratory do when using the P026B probe set. We run GeneMapper on another computer than the one that is controlling the ABI.

1.2.1 On the a computer having GeneMapper to analyse files produced on e.g. an ABI3130XL the cytogeneticist:

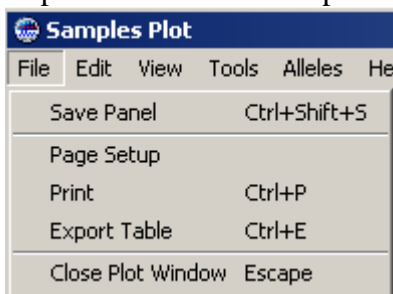
- 1) Analyses the samples, checks the quality and prints the electropherograms of the actual run by use of the GeneMapper program.
- 2) Exports the fragment peak data (sizing tables) by marking the analysed samples (become blue when marked), and clicks at “Display Plots”.

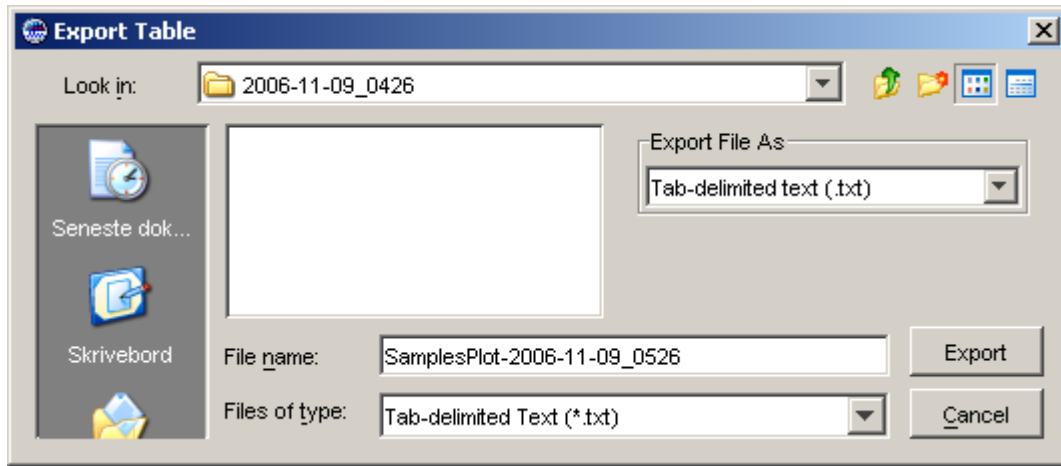


- 3) Ensures the Sizing Table is shown, and if not clicks at the Sizing Table icon:



- 4) Exports the selected samples to the USB-Memory via Export Table at the File menu:





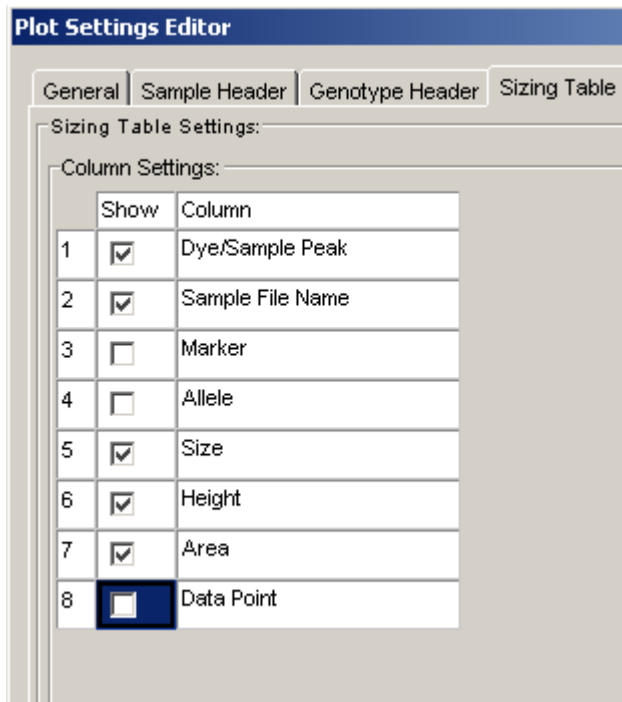
Hints:

It might be smartest to export all samples of the same MLPA probe set of a run to one single file, because RH-MLPA-Analysis does only analyse one kind of MLPA probe sets at a time. I.e. if you both have analysed samples by P095 and by P026B in the same run, then the P095 samples should be exported to one file and the P026B samples to another file.

It might be a good habit in the filename for the exported table to mention the MLPA kit name (if samples are selected from only one kit among others), run number and date.

Export requirements for RH_MLPA-Analysis:

The data fields have to be “Tab-delimited”, and at least the marked fields should be exported. (Note: The MRC Coffalyser also needs Data Point, so include it if you want the file to be valid for Coffalyser too.)

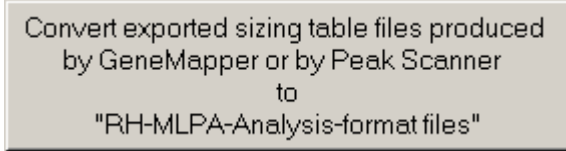


1.2.2 On another computer having Microsoft Access 2000 (or higher) installed the cytogeneticist:

- 1) Starts the program RH-MLPA-Analysis.mdb.
- 2) Selects the appropriate MLPA kit:

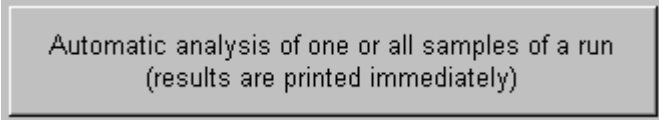


- 3) Clicks at the GeneMapper conversion button:



And then you continue to convert your data like described in “2.2.2 Automatic analysis and print of GeneMapper produced examples” in “instructions-demo.pdf”. The only difference is that you now convert your own data supplied on e.g. an USB-memory instead of converting the demonstration file.

If you have set up the conversion options so that the system remembers where it placed the last converted data then the analysis goes like this when the cytogeneticist:



- 1) Selects the button.
- 2) 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 F: drive is an USB-Memory)

Program version

P026B-vs01 SOTOS kitt (lot 1005) 4.85

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
F:\GeneMapperFragmentData

MLPA-data folder
SamplesPlot-2006-11-09_0526-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



2 Training the analysis program with your own data

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

3 File formats

Here we just illustrate the file formats by showing the contents of the files that are supplied for demonstrating how to convert exported GeneMapper data.

3.1 The format of an exported GeneMapper sample plot sizing table data file

The columns are separated by a tabulator character and the header describing the contents of the columns is included when GeneMapper exports a Sizing Table as a Tab-delimited file. RH-MLPA-Analysis reads the header to pick up the right columns, therefore you are allowed to export more columns than shown in the example, that just contains the necessary columns.

The start of the supplied example file

“C:\RH-MLPA-v483\P026B-vs01-examples-GeneMapper/SamplesPlot-2006-11-09_0526.txt” looks like this:

Dye/Sample Peak	Sample File Name	Size	Height	Area
"B,1"	P026B-case_1_E05_2006-11-09.fsa		121	657
"B,2"	P026B-case_1_E05_2006-11-09.fsa		370	4470
"B,3"	P026B-case_1_E05_2006-11-09.fsa		300	2286
"B,4"	P026B-case_1_E05_2006-11-09.fsa		233	1730
"B,5"	P026B-case_1_E05_2006-11-09.fsa6.5		370	1129
"B,6"	P026B-case_1_E05_2006-11-09.fsa7.86		188	1125
"B,7"	P026B-case_1_E05_2006-11-09.fsa11.08		657	5021
"B,8"	P026B-case_1_E05_2006-11-09.fsa12.22		652	6218
"B,9"	P026B-case_1_E05_2006-11-09.fsa13.47		275	2896
"B,10"	P026B-case_1_E05_2006-11-09.fsa18.46		6589	107599
"B,11"	P026B-case_1_E05_2006-11-09.fsa24.29		176	861
"B,12"	P026B-case_1_E05_2006-11-09.fsa25.43		114	564
"B,13"	P026B-case_1_E05_2006-11-09.fsa27.62		406	3439
"B,14"	P026B-case_1_E05_2006-11-09.fsa30.84		387	1814
"B,15"	P026B-case_1_E05_2006-11-09.fsa32.71		525	5871
"B,16"	P026B-case_1_E05_2006-11-09.fsa42.7		134	1256
"B,17"	P026B-case_1_E05_2006-11-09.fsa55.07		1048	11977
"B,18"	P026B-case_1_E05_2006-11-09.fsa82.32		1942	29243
"B,19"	P026B-case_1_E05_2006-11-09.fsa91.89		3196	21346
"B,20"	P026B-case_1_E05_2006-11-09.fsa127.33		3325	21652
"B,21"	P026B-case_1_E05_2006-11-09.fsa133.48		3587	23522
"B,22"	P026B-case_1_E05_2006-11-09.fsa141.25		4072	26045
"B,23"	P026B-case_1_E05_2006-11-09.fsa146.28		4846	30762
"B,24"	P026B-case_1_E05_2006-11-09.fsa152.72		5026	32499
"B,25"	P026B-case_1_E05_2006-11-09.fsa158.86		3118	19537
"B,26"	P026B-case_1_E05_2006-11-09.fsa166.01		3783	24353
"B,27"	P026B-case_1_E05_2006-11-09.fsa171.79		4599	30902
"B,28"	P026B-case_1_E05_2006-11-09.fsa177.14		4345	29224
"B,29"	P026B-case_1_E05_2006-11-09.fsa185.09		4176	28163
"B,30"	P026B-case_1_E05_2006-11-09.fsa192.91		3957	26116
"B,31"	P026B-case_1_E05_2006-11-09.fsa201.29		4016	27694
"B,32"	P026B-case_1_E05_2006-11-09.fsa210.31		372	2117
"B,33"	P026B-case_1_E05_2006-11-09.fsa211.37		1668	11318
"B,34"	P026B-case_1_E05_2006-11-09.fsa220.52		2430	16396
"B,35"	P026B-case_1_E05_2006-11-09.fsa229.16		2221	14634
"B,36"	P026B-case_1_E05_2006-11-09.fsa239.62		2863	19421

"B,37"	P026B-case_1_E05_2006-11-09.fsa248.35	1658	11540	
"B,38"	P026B-case_1_E05_2006-11-09.fsa258.12	2354	17005	
"B,39"	P026B-case_1_E05_2006-11-09.fsa266.48	975	6991	
"B,40"	P026B-case_1_E05_2006-11-09.fsa273.44	547	3594	
"B,41"	P026B-case_1_E05_2006-11-09.fsa274.36	1474	10815	
"B,42"	P026B-case_1_E05_2006-11-09.fsa283.13	1408	10175	
"B,43"	P026B-case_1_E05_2006-11-09.fsa291.25	1936	14227	
"B,44"	P026B-case_1_E05_2006-11-09.fsa298.82	154	922	
"B,45"	P026B-case_1_E05_2006-11-09.fsa299.73	1013	7516	
"B,46"	P026B-case_1_E05_2006-11-09.fsa310.34	1763	13370	
"B,47"	P026B-case_1_E05_2006-11-09.fsa319.4	1203	9441	
"B,48"	P026B-case_1_E05_2006-11-09.fsa328.91	1539	12824	
"B,49"	P026B-case_1_E05_2006-11-09.fsa337.48	568	4405	
"B,50"	P026B-case_1_E05_2006-11-09.fsa346.0	592	4963	
"B,51"	P026B-case_1_E05_2006-11-09.fsa354.49	1315	11210	
"B,52"	P026B-case_1_E05_2006-11-09.fsa364.19	1756	14968	
"B,53"	P026B-case_1_E05_2006-11-09.fsa375.13	967	8199	
"B,54"	P026B-case_1_E05_2006-11-09.fsa383.02	1512	12694	
"B,55"	P026B-case_1_E05_2006-11-09.fsa391.34	885	7965	
"B,56"	P026B-case_1_E05_2006-11-09.fsa399.72	1380	12103	
"B,57"	P026B-case_1_E05_2006-11-09.fsa407.75	1210	10872	
"B,58"	P026B-case_1_E05_2006-11-09.fsa417.32	545	5015	
"B,59"	P026B-case_1_E05_2006-11-09.fsa426.45	1091	10250	
"B,60"	P026B-case_1_E05_2006-11-09.fsa435.03	766	7251	
"B,61"	P026B-case_1_E05_2006-11-09.fsa444.28	504	4799	
"B,62"	P026B-case_1_E05_2006-11-09.fsa453.19	1343	12717	
"B,63"	P026B-case_1_E05_2006-11-09.fsa460.77	873	8608	
"G,1"	P026B-case_1_E05_2006-11-09.fsa18.46	580	2304	
"G,2"	P026B-case_1_E05_2006-11-09.fsa141.72	87	802	
"G,3"	P026B-case_1_E05_2006-11-09.fsa143.36	61	765	
"G,4"	P026B-case_1_E05_2006-11-09.fsa144.42	61	628	
"G,5"	P026B-case_1_E05_2006-11-09.fsa145.47	58	468	
"G,6"	P026B-case_1_E05_2006-11-09.fsa172.19	95	775	
"G,7"	P026B-case_1_E05_2006-11-09.fsa173.1	59	510	
"G,8"	P026B-case_1_E05_2006-11-09.fsa174.11	58	779	
"G,9"	P026B-case_1_E05_2006-11-09.fsa175.22	56	627	
"G,10"	P026B-case_1_E05_2006-11-09.fsa176.33	56	483	
"Y,1"	P026B-case_1_E05_2006-11-09.fsa30.94	2979	14587	
"R,1"	P026B-case_1_E05_2006-11-09.fsa15.76	72	721	
"R,2"	P026B-case_1_E05_2006-11-09.fsa19.3	281	1985	
"R,3"	P026B-case_1_E05_2006-11-09.fsa20.75	665	4474	
"R,4"	P026B-case_1_E05_2006-11-09.fsa28.14	64	356	
"R,5"	P026B-case_1_E05_2006-11-09.fsa30.94	9185	96026	
"R,6"	P026B-case_1_E05_2006-11-09.fsa39.37	399	5173	
"R,7"	P026B-case_1_E05_2006-11-09.fsa52.37	397	2410	
"R,8"	P026B-case_1_E05_2006-11-09.fsa76.6	377	2115	
"R,9"	*" P026B-case_1_E05_2006-11-09.fsa100.0	415	2250	
"R,10"	*" P026B-case_1_E05_2006-11-09.fsa139.0	455	2503	
"R,11"	*" P026B-case_1_E05_2006-11-09.fsa150.0	461	2513	
"R,12"	*" P026B-case_1_E05_2006-11-09.fsa160.0	464	2575	
"R,13"	*" P026B-case_1_E05_2006-11-09.fsa200.0	481	2719	
"R,14"	*" P026B-case_1_E05_2006-11-09.fsa250.0	473	2878	
"R,15"	*" P026B-case_1_E05_2006-11-09.fsa300.0	510	3220	
"R,16"	*" P026B-case_1_E05_2006-11-09.fsa340.0	522	3518	
"R,17"	*" P026B-case_1_E05_2006-11-09.fsa350.0	532	3599	
"R,18"	*" P026B-case_1_E05_2006-11-09.fsa401.6	548	4003	
"R,19"	*" P026B-case_1_E05_2006-11-09.fsa450.0	546	4305	
"R,20"	*" P026B-case_1_E05_2006-11-09.fsa490.0	547	4544	
"R,21"	*" P026B-case_1_E05_2006-11-09.fsa500.0	543	4595	
"B,1"	P026B-case_2_A05_2006-11-09.fsa		437	6098
"B,2"	P026B-case_2_A05_2006-11-09.fsa		344	2721
"B,3"	P026B-case_2_A05_2006-11-09.fsa	0.75	269	2099
"B,4"	P026B-case_2_A05_2006-11-09.fsa	6.56	176	1222
"B,5"	P026B-case_2_A05_2006-11-09.fsa	8.47	250	1532

...

etc (Note: Only all data of the first sample and some few lines of the second sample are shown)

3.2 The format of an RH-MLPA-Analysis format file

These files have same format as GeneScan tab delimited "export format files", except that the columns "Minutes" and "Data Point" may be replaced by something else that serve as place holders. E.g. when converting GeneMapper data then the actual "Sample file name" is repeated in the column "Minutes". (MLPA analysis doesn't use the columns Minutes and Data Point, so you can place any text in these fields. MLPA analysis only needs the B-peaks.).

Each file does only contain data for one sample and there is no header describing the contents of the 6 columns that are separated by a tabulator character.

After you do the conversion of the supplied file the start and end of the supplied example file (P026B-case_1): "C:\RH-MLPA-v483\ P026B-vs01-examples-GeneMapper-Peak_Scanner/SamplesPlot-2006-11-09_0526-RH-MLPA/ P026B-case_1_E05_2006-11-09.txt" looks like this:

Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point	This header is not part of the file
B,1	P026B-case_1_E05_2006-11-09		121	657	0	
B,2	P026B-case_1_E05_2006-11-09		370	4470	0	
B,3	P026B-case_1_E05_2006-11-09		300	2286	0	
B,4	P026B-case_1_E05_2006-11-09		233	1730	0	
B,5	P026B-case_1_E05_2006-11-09	6.5	370	1129	0	
B,6	P026B-case_1_E05_2006-11-09	7.86	188	1125	0	
B,7	P026B-case_1_E05_2006-11-09	11.08	657	5021	0	
B,8	P026B-case_1_E05_2006-11-09	12.22	652	6218	0	
B,9	P026B-case_1_E05_2006-11-09	13.47	275	2896	0	
B,10	P026B-case_1_E05_2006-11-09	18.46	6589	107599	0	
B,11	P026B-case_1_E05_2006-11-09	24.29	176	861	0	
B,12	P026B-case_1_E05_2006-11-09	25.43	114	564	0	
B,13	P026B-case_1_E05_2006-11-09	27.62	406	3439	0	
B,14	P026B-case_1_E05_2006-11-09	30.84	387	1814	0	
B,15	P026B-case_1_E05_2006-11-09	32.71	525	5871	0	
B,16	P026B-case_1_E05_2006-11-09	42.7	134	1256	0	
B,17	P026B-case_1_E05_2006-11-09	55.07	1048	11977	0	
B,18	P026B-case_1_E05_2006-11-09	82.32	1942	29243	0	
B,19	P026B-case_1_E05_2006-11-09	91.89	3196	21346	0	
B,20	P026B-case_1_E05_2006-11-09	127.33	3325	21652	0	
B,21	P026B-case_1_E05_2006-11-09	133.48	3587	23522	0	
B,22	P026B-case_1_E05_2006-11-09	141.25	4072	26045	0	
B,23	P026B-case_1_E05_2006-11-09	146.28	4846	30762	0	
B,24	P026B-case_1_E05_2006-11-09	152.72	5026	32499	0	
B,25	P026B-case_1_E05_2006-11-09	158.86	3118	19537	0	
...						
G,9	P026B-case_1_E05_2006-11-09	175.22	56	627	0	
G,10	P026B-case_1_E05_2006-11-09	176.33	56	483	0	
Y,1	P026B-case_1_E05_2006-11-09	30.94	2979	14587	0	
R,1	P026B-case_1_E05_2006-11-09	15.76	72	721	0	
R,2	P026B-case_1_E05_2006-11-09	19.3	281	1985	0	
R,3	P026B-case_1_E05_2006-11-09	20.75	665	4474	0	
R,4	P026B-case_1_E05_2006-11-09	28.14	64	356	0	
R,5	P026B-case_1_E05_2006-11-09	30.94	9185	96026	0	
R,6	P026B-case_1_E05_2006-11-09	39.37	399	5173	0	
R,7	P026B-case_1_E05_2006-11-09	52.37	397	2410	0	
R,8	P026B-case_1_E05_2006-11-09	76.6	377	2115	0	
R,9	P026B-case_1_E05_2006-11-09	100.0	415	2250	0	
R,10	P026B-case_1_E05_2006-11-09	139.0	455	2503	0	
R,11	P026B-case_1_E05_2006-11-09	150.0	461	2513	0	
R,12	P026B-case_1_E05_2006-11-09	160.0	464	2575	0	
R,13	P026B-case_1_E05_2006-11-09	200.0	481	2719	0	
R,14	P026B-case_1_E05_2006-11-09	250.0	473	2878	0	
R,15	P026B-case_1_E05_2006-11-09	300.0	510	3220	0	
R,16	P026B-case_1_E05_2006-11-09	340.0	522	3518	0	
R,17	P026B-case_1_E05_2006-11-09	350.0	532	3599	0	
R,18	P026B-case_1_E05_2006-11-09	401.6	548	4003	0	
R,19	P026B-case_1_E05_2006-11-09	450.0	546	4305	0	
R,20	P026B-case_1_E05_2006-11-09	490.0	547	4544	0	
R,21	P026B-case_1_E05_2006-11-09	500.0	543	4595	0	