

## GeneMarker® for MLPA Analysis Using Luminex xMap® Technology

### Introduction

MLPA has proven to be an important technique in the detection of exon copy number changes associated with breast and colon cancers, as well as trisomies found in Down Syndrome (3, 4, 5). Coupling MLPA with Luminex microsphere technology has the potential to also be as important in the detection of copy number changes associated with Niemann-Pick type C (6, 7, 8), alpha-thalassemia (8) and other genetic diseases. Accurate analysis of Luminex-microsphere flow cytometry instrument generated data is critical to disease diagnosis.

SoftGenetics' GeneMarker now includes a Luminex-MLPA module that quickly and accurately analyzes data from Luminex instruments (Luminex 100 and Luminex 200). The software is compatible with the Luminex instrument data format (\*.csv).

GeneMarker® Luminex-MLPA analysis automatically performs background subtraction, and flags suspect intensities according to user-specified thresholds. The software selects the least variable sample, from the sample set to act as the reference for determining copy number change within the patient sample set.

### Procedure

Analysis of Luminex MLPA data is easy to perform. In the menu bar of GeneMarker click Tools and select 'Luminex MLPA Analysis' from the drop-down menu. Select and open the file to analyze. The Luminex MLPA Data window will activate (figure 1). The user may select their own control probe or normal sample (right click on the sample row or cell to activate the control edit menu) or bead count threshold. Click OK to initiate analysis. GeneMarker will automatically perform background subtraction, select control sample and calculate copy number changes.

Ratios are automatically calculated and displayed in graphical and tabular forms. Default settings can easily be adjusted to meet user specified requirements.

In addition to reading Luminex data, GeneMarker® can perform fragment analysis and genotyping on four or five color data sets from any slab gel or capillary electrophoresis system. This software automatically corrects for many common problems—instrument spike, color pull-up, peak pull-up, noisy data, saturated peaks and stutter peaks—saving significant analysis time and cost, efficiently analyzing raw fragment data within seconds. GeneMarker is robust software to analyze DNA fragment data labeled with MegaBACE™ dyes (Amersham), Big Dye® (Applied Biosystems Inc.) or Beckman dyes from a variety of platforms: ABI DNA Analyzer or Genetic Analyzer [SoftGenetics is a member of the Applied BioSystems Software Community Program ([http://www.appliedbiosystems.com/support/software\\_community/software\\_by\\_other\\_companies.cfm](http://www.appliedbiosystems.com/support/software_community/software_by_other_companies.cfm))], Amersham instruments or Beckman instruments. GeneMarker is compatible with files from all major capillary and slab gel electrophoresis systems including ABI files (\*.FSA, \*AB1, \*.ABI), SCF files, MegaBACE® files (\*.RSD, \*.ESD), SpectruMedix files (\*.SMD, \*.SMR), Beckman files and Li-Cor files (\*.scf). The software features high efficiency allele calling, adjustable parameters and a variety of reporting options.

Location	Sample	alpha1E3	alpha2E1	alpha2E3	BCAF3	CTNNB	DMD	HBB-E1	HBB-E2
1	B1	477951	439.0	0.0	0.0	0.0	0.0	0.0	0.0
2	B1	477951	439.0	0.0	0.0	0.0	0.0	0.0	0.0
3	C1	477951	389.0	0.0	0.0	0.0	0.0	0.0	0.0
4	D1	474348	429.0	0.0	0.0	0.0	0.0	0.0	0.0
5	E1	474348	450.0	0.0	0.0	0.0	0.0	0.0	0.0
6	F1	481204	59.0	248.0	87.0	247.0	105.0	93.5	223.5
7	G1	481204	47.5	249.0	90.0	271.0	102.0	89.0	223.5
8	H1	H1_M1	453.5	1310.0	758.0	1589.5	913.0	326.0	1458.0
9	A2	A2_M1	385.5	1138.0	665.0	1299.0	645.0	277.5	1313.0
10	B2	B2_F1	419.0	1229.5	675.5	1395.0	732.0	498.0	1324.0
11	C2	C2_F2	813.0	1957.0	807.0	1720.0	936.0	601.0	1957.0
12	D2	D2_F3	525.0	1384.5	805.0	1700.0	884.0	573.0	1535.0
13	E2	E2_F4	488.0	1306.5	767.5	1707.0	863.0	654.0	1501.0
14 (B)	F2	F2_00000	27.0	32.0	156.0	77.5	16.0	35.5	123.0

Figure 1. Luminex MLPA Data analysis window: The top field displays instrument and project information. The bottom field lists samples and genes analyzed in the project (control sample edit menu is active), notice sample 14 has been selected as the background (B) sample. The lower left corner contains the Suspect bead Count dialog box (the default setting value is 100).

### Results

After background subtraction, reference sample selection and intensity ratio calculation, the data are presented in an intensity ratio plot. Sample number 8, H1\_M1, was selected as the reference, designated by the # sign. Probes with a normal copy number are indicated by green squares. Probes that may have a duplication or deletion are indicated by red squares (figure 2).

Sample C1 (highlighted in the list of samples and indicated in red above the intensity ratio plot) is from a female patient. The DMD ratio is 1.699: one copy of the x-linked DMD gene in reference sample H1\_M1 and two copies of the DMD gene in sample C1. The intensity of DMD is highlighted in blue in the intensity ratio table, the peak corresponding to DMD in the comparison chart is highlighted in light blue and the intensity ratio of DMD is indicated by the yellow square (red if not selected) in the ratio plot. The three red squares in the intensity ratio plot clearly indicate a deletion of one copy of HBB exons 1, 2 and 3 (figure 2).

Samples in the MLPA analysis window that contain probes that did not meet the minimum bead threshold (Suspect Count) will be identified (Figure 3). Suspect probes will be labeled in red in the peak comparison chart and the corresponding point in the intensity ratio plot will be pink.

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Figure 2. MLPA analysis window displaying list of patient samples (left), peak height comparison chart (top center: red peaks are control sample; blue peaks are patient sample), intensity ratio plot comparing patient sample to control sample (bottom center) and intensity ratio table (upper right).

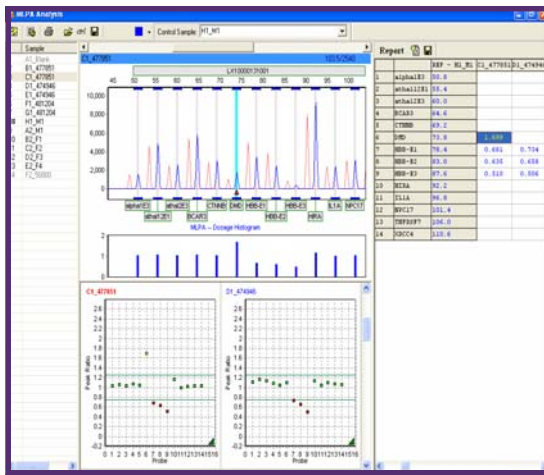
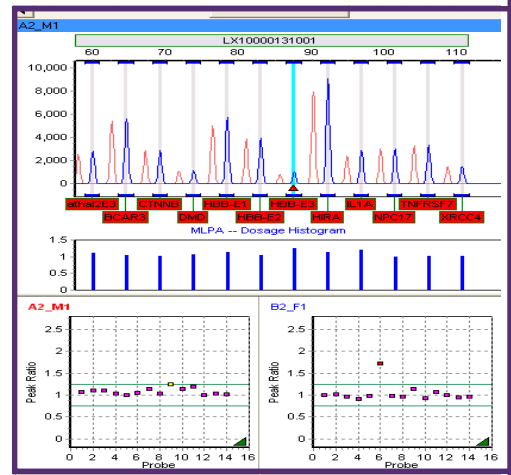


Figure 3. MLPA analysis window showing peak height comparison chart (labels are in red) and intensity ratio plot (squares are labeled in pink for suspect probes that contained too few beads)



GeneMarker for Luminex MLPA converts Luminex bead intensities into easy-to-read chromatograms.

## Reporting

The intensity ratio table can be saved as a tab delimited \*.txt file. A patient report (Figure 4) can be printed or saved as \*.MDI or \*.jpg formats.

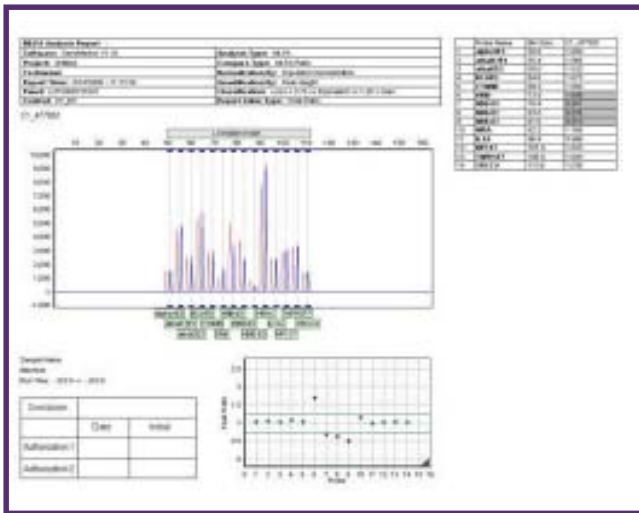


Figure 4. Patient report includes sample ID, analysis parameters, intensity ratio report (copy number changes are shaded), intensity ratio plot and peak height comparison chart by individual sample.

## Discussion

Various techniques including DGGE (Denaturing Gradient Gel Electrophoresis), DHPLC (Denaturing High Performance Liquid Chromatography) and SSCA (Single Strand Confirmation Analysis) effectively identify SNPs and small insertions and deletions. MLPA is one of the only accurate, time efficient techniques to detect genomic deletions and insertions which are frequent causes of cancer and genetic diseases. Luminex-MLPA can successfully and with high sensitivity easily determine the relative copy number of exons within a gene. GeneMarker provides a quick and easy-to-use tool for necessary quantification and reporting of the data.

### References

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