MLPA® Analysis Using GeneMarker® Software

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Introduction

Multiplex Ligation-Dependent Probe Amplification (MLPA®) is a PCR-based technique developed by MRC-Holland. Since its introduction in 2002, it has become a widely-used and effective technique for detecting copy number variations associated with many common diseases and cancers[1, 2, 3]. MLPA is simpler, more senstitive, and less time intensive when compared to other traditional techniques for detecting CNVs, including PCR amplification, FISH, and Southern blotting.

Various software packages exist for the analysis of MLPA data. Unfortunately, these programs tend to be difficult to use, have limited reporting capabilities, or require tedious navigation through a spreadsheet-like interface. Furthermore, these programs typically provide no way of actually processing and normalizing the raw data - necessitating a *second* software package for that purpose.

GeneMarker software's integrated MLPA analysis application provides a solution to these problems. GeneMarker software processes and normalizes raw datafiles, allowing the user to load their raw data, process the data, and proceed directly to MLPA analysis without any time consuming and error-prone data transfers. GeneMarker is easy to use, thanks to an interactive user-interface with linked navigation (figure 1). GeneMarker also provides several informative reporting options, which may be saved electronically or printed directly (figure 4). Finally, Quality Control features new to GeneMarker 2.6.0 automatically detect quality control fragments and alert the user to potentially compromised runs (figure 3).
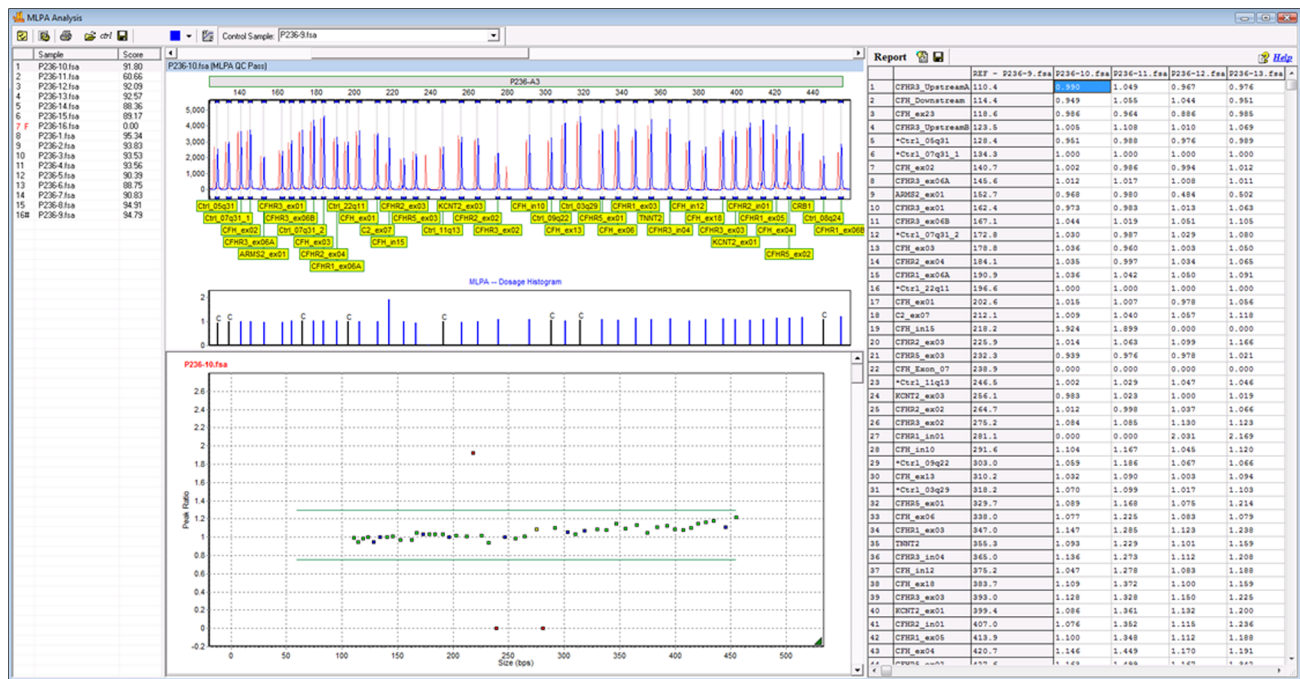

Figure 1: GeneMarker's MLPA analysis screen. The trace comparison (top, middle) shows a direct comparison between the selected sample and the reference. The resulting peak ratios are plotted in the ratio plot below, and are reported for each sample in the Report Table to the right. (In this example there is one homozygous duplication, and two homozygous deletions.)

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Procedure

1. Import Raw Data.
2. Navigate to Project > Run.
3. Select the Panel and Size Standard, and Select MLPA as the Analysis type. Or, select the appropriate pre-saved template (figure 2).
4. In the final page of the Run Wizard, choose from the normalization options and if desired, activate automated quality control. Click OK.
5. Review the processed data.
6. Proceed to MLPA analysis (Applications > MLPA analysis).
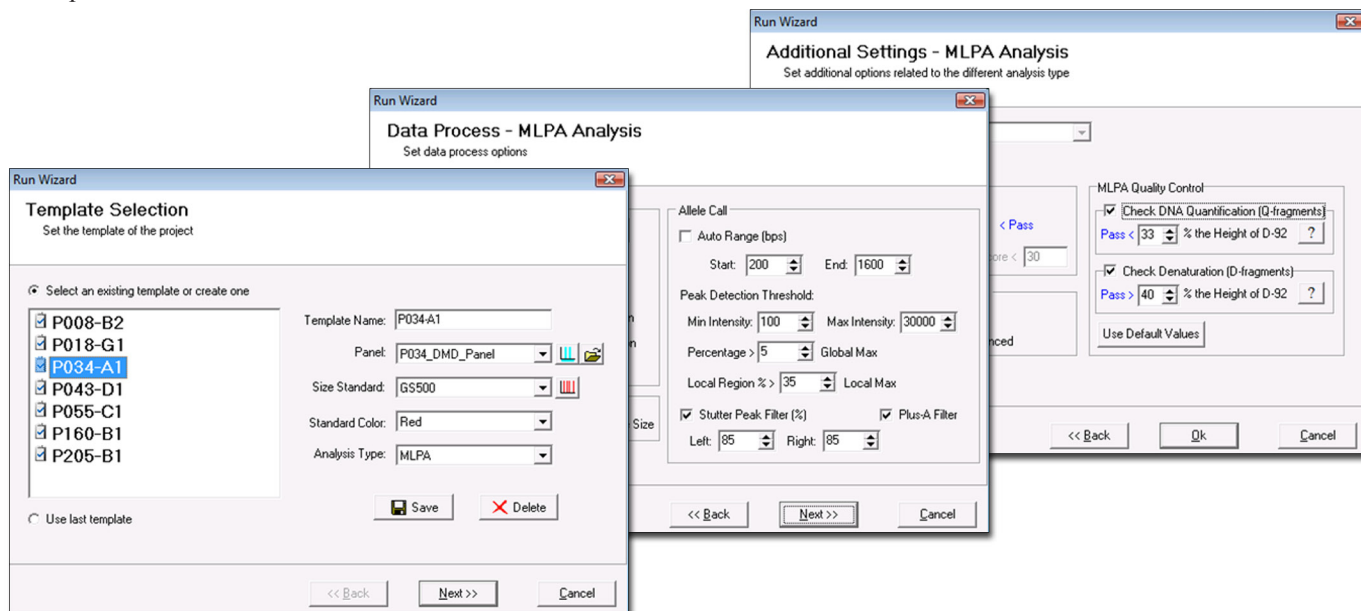7. Export or Save the results.

Figure 2: The user may adjust and customize their analysis settings using the three pages of the "Run Wizard". Settings may be saved as templates, allowing the user to process their samples by simply selecting the appropriate template and clicking Next > Next > OK.
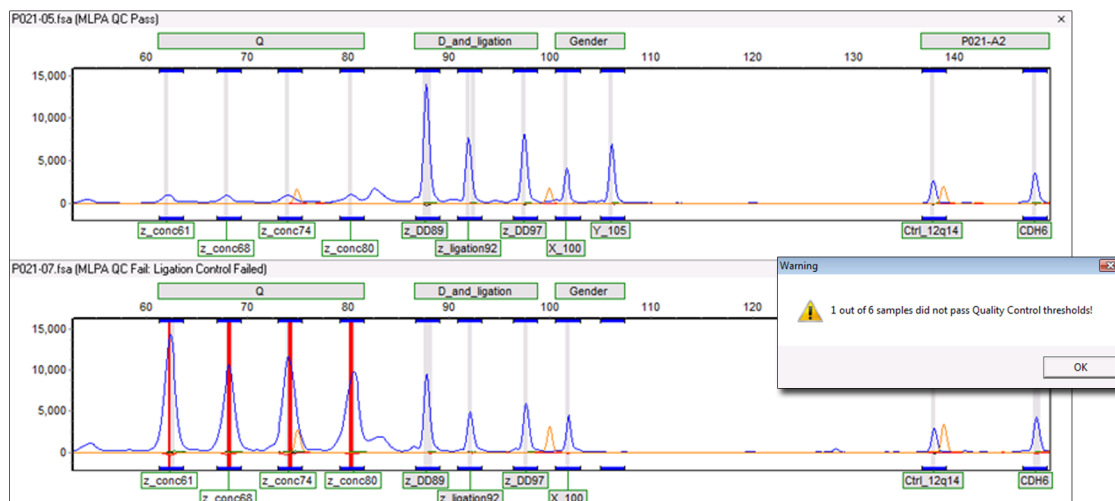

Figure 3: An example of a normal MLPA run (above) and a sample which has unusually high Q fragments indicative of low DNA quantity (below). GeneMarker's new settings automatically detect these fragments and flag any offending peaks.

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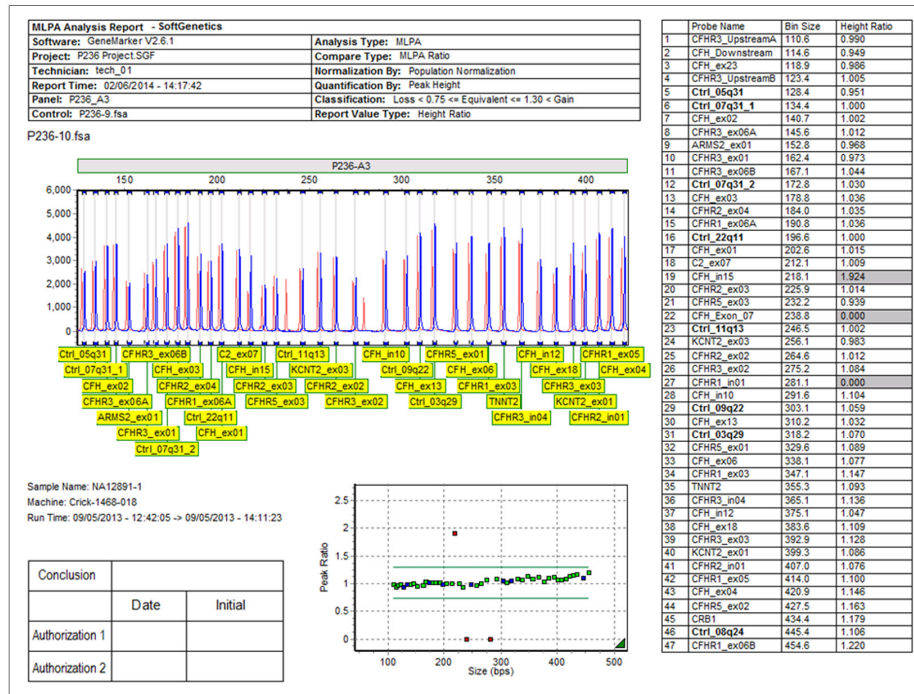
Results & Discussion

Figure 4: A typical MLPA report. The report contains a customizable header with project and user information, the trace comparison, ratio plot, validation box, and relevant excerpt from the Report Table. Reports may be printed directly or saved electronically.

MLPA analysis using GeneMarker is accurate, rapid, and has been independently validated by labs throughout the world[4]. Compared to other available programs, GeneMarker is easier to use, has more flexible reporting options, and eliminates the need to use two or more programs for MLPA analysis - instead, all of the necessary features are combined into a single program.

GeneMarker also comes fully equipped with a panel editor tool. This tool allows the user to easily save, manage, and calibrate panels as needed. Panels and other important files may be shared over a local network, allowing institutions to centralize important panel XML files, projects, and other files. GeneMarker is also preloaded with an extensive user management system, and several other tools with clinical research applications, including Fragile X, MSI, and Aneuploidy analysis. GeneMarker is compatible with all major CE instruments, kits, and custom chemistries.

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