

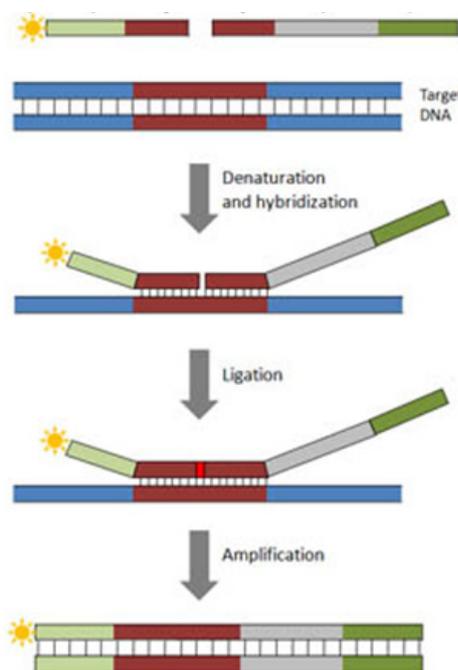
MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION ANALYSIS (MLPA)

What is MLPA?

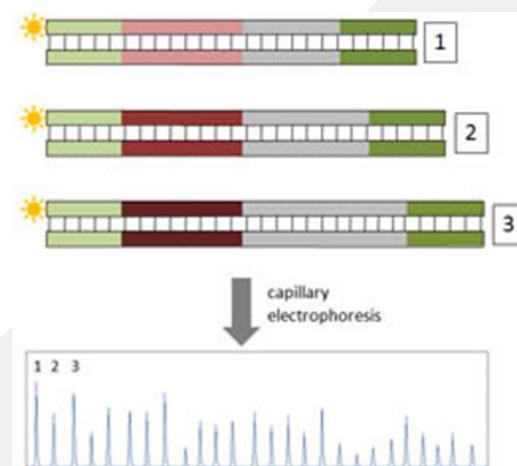
Multiplex Ligation-dependent Probe Amplification (MLPA), a registered trademark of MRC-Holland, is a relatively simple and robust multiplex PCR method for detecting chromosomal DNA copy number changes in multiple targets.

As the name suggests, amplification of a probe is made dependent on a ligation step, which can only occur if target DNA is present in the sample. This is achieved as follows. For a short sequence of target DNA, two adjacent probes are designed that contain the forward and reverse primer sequence, respectively. In addition, one of both probes contains a stuffer sequence of which the length can be varied in function of the experiment. The probes are hybridized against the target DNA and subsequently ligated. Only if ligation happened, a functional PCR strand appears, so that amplification only happens if target DNA is present in the sample.

The amount of PCR product is proportional to the amount of target DNA present in the sample, making the technique suitable for quantitative measurements (relative ploidy). Multiple probe pairs are pooled and amplified with the same primer pair, so that the technique does not suffer from typical limitations of multiplex PCR.



The forward primer is fluorescently labeled so that the amplicons can be visualized using capillary electrophoresis.



The stuffer sequences are varied in length so that a range of up to 50 targets can be amplified and separated in a single experiment.

YOUR ADVANTAGES



WHY USE **BioNumerics** FOR YOUR MLPA ANALYSIS?

- ✓ Automated import and preprocessing of curves
- ✓ Easy setup of experiments
- ✓ Automated assignment and scoring
- ✓ Detailed plots and reports



Automated import/preprocessing of curves



The BioNumerics MLPA plugin provides an automated workflow for preprocessing of MLPA profiles, starting from raw sequencer chromatogram files or preprocessed peak tables (Applied Biosystems and Beckman). Multiple probe mixes can be combined and for each probe mix, the MLPA setup can be entered initially in the software. This includes the dye setup, the strategy for automated name and information parsing, and selection of the size standard used.

Automated assignment and scoring



Peaks on the samples are assigned automatically to known probes based upon the size information provided in the probe mix definitions. Depending on the instrument, a one-time correction between "observed size" and "theoretical size" might be required for some probes. The plugin provides a function to redefine probe sizes according to the center position of all observed peaks.

The plugin utilizes an iterative algorithm to normalize the peak height or area in two dimensions: (1) all peaks within a profile (median, mean, linear or exponential) and (2) the same probe across all samples (median, mean and minimum). Peak scoring can be calculated from the normalized quantifications or from dosage quotients. Scored probes can be sorted according to their appearance on the chromosomes so that it is easy to assess potential large deletions or duplications.

If manual inspection indicates that the scoring parameters need to be adjusted, the settings dialog box allows different scoring strategies to be utilized and loss-gain values to be adjusted. All settings can be saved into analysis templates.

NOTE:

The MLPA plugin is a free add-on. The minimum configuration for installation of the plugin includes the "Fingerprint Data" and "Character Data" modules. Please contact us for more information.

Easy setup of experiments

The MLPA plugin allows probe mix definitions to be imported using the format provided by MRC Holland. For existing probe mixes, probes can be added, deleted or edited. In a design setup, new custom probe mixes can be created as well.

The default scoring categories Undefined, Absent, Loss, Normal, Gain and Amplification can be edited. Since the automatic assignment of scores relies on normalization and comparison with known reference samples, the inclusion of control samples is important. The MLPA plugin uses negative controls (all normal) and positive controls with known aberrations.

Detailed plots and reports

A more in-depth analysis of the quantification distribution for all probes together or each probe individually can be obtained in the probe plot distribution window. The plot statistics can be saved into the settings for more accurate automatic scoring.

The quality of the experiments can be monitored using the Quality control window which allows various parameters to be checked for a run or selection of samples. The thresholds for "good/bad" indication can be set by the user.

Finally, detailed reports can be generated for all probes of a selected sample in the database. The information and properties to be displayed can be chosen.



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