MRC Holland Support

What are the main advantages and limitations of MLPA?

Advantages

The main advantages of MLPA are that it is a high throughput and cost effective way to check for duplications and deletions, and a large number of products are available.

MLPA is a multiplex reaction, meaning one reaction provides information on up to 60 targets, as opposed to conventional PCR where you would need 60 independent reactions. For most applications a single MLPA reaction is sufficient to answer the specific questions asked by a physician or researcher.

MLPA reactions are cost-effective. The current price (2024) for 100 MLPA reactions is € 1416. This price includes all reagents (probemix, buffers, ligase, polymerase, dNTPs and labelled PCR primers). What also adds to the affordability of MLPA is that all MLPA assays use the same reagent kit. In addition, the necessary equipment for MLPA consists of a capillary electrophoresis device and thermocycler, both of which are already present in most molecular biology laboratories.

Another advantage of MLPA is that it can be performed on a large number of samples simultaneously. The assay is reproducible, easy to perform, and sensitive. Each MLPA reaction requires only 50 ng of human DNA, can distinguish sequences differing by a single nucleotide, and can detect small copy number differences—e.g. 3 vs. 2 copies of a given gene sequence in a DNA sample. Each MLPA probe detects a sequence of approximately 60-80 nucleotides, meaning that single exon deletions and duplications can be detected.

Limitations

While being a very robust technique there are some limitations of MLPA: its sensitivity to contaminants, its inability to detect anomalies at the single cell level, its inability to detect unknown SNVs, and its sensitivity to novel benign SNVs at or near a probe ligation site. Note that some of these limitations are trivial and that they can often be addressed by taking appropriate precautions.

MLPA reactions are more sensitive to contaminants than conventional PCR reactions. Examples of contaminants that may affect MLPA reactions are small remnants of phenol and high levels of salts. Contaminants influencing your MLPA reactions can generally be easily detected in your MLPA results through examination of the probemix quality control fragments and warnings issued by the Coffalyser.Net analysis software.

MLPA cannot be used to investigate single cells. This is due to the limitation on the amount of DNA needed for an MLPA reaction.

MLPA analysis of DNA samples from cell mixtures will give the average copy number per cell. In the case of tumour analysis, it can be difficult to detect copy number variations if the sample from which the DNA was derived contained less than 50% tumour cells.

MLPA is primarily a method that identifies genomic deletions/duplications. MLPA is not a suitable method to detect *unknown* SNVs. However, MLPA is able to detect *known* point SNVs. Probes can be designed such that the ligation site is located directly at the location of the SNV. Ligation will then only occur on non-mutated sequences, and when the SNV is present this will result in a decreased probe signal.

MLPA probes are not only sensitive to complete deletions of a probe's target sequence, but also to small deletions, insertions, and mismatches. Any mismatch in the probe's target site can theoretically affect the probe's signal. The strength of the destabilising effect depends on the location of the mismatch, the type of mismatch, and the Tm (melting temperature) of the probe's hybridising sequence. This means that a non-pathogenic SNV can also result in a decreased probe signal, possibly leading to an ambiguous result or in the worst case mimicking a deletion. When designing MLPA probes, we try to exclude any sequences where known SNVs are found using the latest genetic search engines. However, this isn't always possible, and new SNVs are continuously being discovered. We recommend confirming all MLPA findings with another method or a

confirmation probemix, especially in the case of single probe deletions.

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