Multiplex ligation-dependent probe amplification (MLPA)

1. Methodology

Multiplex Ligation-dependent Probe Amplification (MLPA) is a multiplex PCR method used to detect abnormal copy numbers of up to 50 different genomic DNA or RNA sequences. Furthermore, MS-MLPA can detect DNA methylation changes. Is the most reliable and cost-effective method of detecting known deletion, duplications, and specific copy number variations (CNVs). Like array CGH, MLPA detects copy number variants and the interpretation of the results can be complicated by naturally occurring copy number variations. The probes or probe kits used have been selected and validated to reduce the likelihood of false positive or negative results.

Copy number variants in targeted regions of the genes were identified by hybridizing with MLPA (Multiplex Ligation-dependent Probe Amplification) probes. Each MLPA probe consists of two hemi-probes that bind to adjacent sites on the target sequence. Upon ligation and subsequent PCR amplification, each distinct MLPA probe (specific to distinct target regions) generates an amplicon with a unique length which are separated and quantified by capillary electrophoresis. Heterozygous deletions within target sequences will prevent efficient probe binding and give a 35-50% reduced relative peak area of the amplification product specific to that probe set. Copy number differences of various exons between test and control DNA samples can be detected by analyzing the MLPA peak patterns.

Multiplex Ligation-dependent Probe Amplification (MLPA®) probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%. MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect most inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region do exist but remain undetected. The MLPA test will not detect point mutations in the tested genes. However, a point mutation or polymorphism can be detected indirectly, because this variation is located at the sequence detected by a probe. SNV presence results in reduced probe binding efficiency, and a reduction in the relative peak area. Therefore, single exon deletions detected by MLPA should always be confirmed by other methods like multiplex PCR or sequencing.

Applications

- Small deletions and rearrangements associated to specific regions, genes or exons
- Specific microdeletion syndromes
- Diseases caused by methylation defects (MS-MLPA)
- Specific Uniparental disomy (UPD)

Limitations

- Balanced chromosomal rearrangements
- Telomeric deletions and duplications
- Deletions and duplications that are not identified by the MLPA probes used
- Point mutations, small insertions and deletions
- Sequence repeats or disorders caused by mutations in mitochondrial DNA

2. Sample requirements and TAT

The following sample types are accepted for Igenomix genetic tests. A thorough labelling of the tube with unique identifying information is suggested, incorrect labelling can lead to rejection of the sample. The minimum required information to identify and accept a sample is: Patient's full name, Date of birth, Gender, Medical Record Number, and Clinical Indication.

Sample type	TAT	Container	Volume	Temperature
Peripheral Blood	2-3 weeks	EDTA tube	3mL	Room temperature
CVS	2-3 weeks	CVS sterile tube either transferred into a sterilized conical tube that contains (RPMI) 1640 media or into a saline solution with 1% antibiotic	300-500 mg of tissue obtained from routine CVS	Room temperature
Amnio	2-3 weeks	Sterilized conical tube sealed with parafilm	15-20 mL Amniotic fluid	Room temperature
Products of Conception	2-3 weeks	Tissue in sterile container in saline Cardiac or cord blood in Vacutainer	1 cm3 (sterile) fetal tissue and/or villi in tissue culture media or Preferred fetal tissue sample sites include buttocks or thigh. If fetal tissue is not available placental villi can be utilized	Room temperature
Extracted DNA	2-3 weeks	In a sealed eppendorf tube	A minimum 1 microgram of DNA at a concentration of 50-100 ng/microliters	Room temperature

*Maternal blood sample must be sent with all products of conception, CVS and Amnio samples