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CGT Laboratory

MLPA

Multiplex Ligation-dipendent Probe Amplification

Introduction

Cogentech's Cancer Genetic Test (CGT Lab) laboratory has developed molecular diagnostic tests to detect and evaluate pathogenic variants that predispose patients and their family members to a higher risk of developing cancer, allowing the oncologist to make timely and accurate diagnoses for better targeting of treatment the patient. Diagnostic tests for different types of hereditary cancers and tests for variants predictive of response to certain therapies, particularly Parp inhibitors, are performed in our laboratories.

These tests involve the use of the Multiplex Ligation-dependent Probe Amplification (MLPA) method for the identification of large deletions or duplications. The method can be used either to confirm data obtained by Next Generation Sequencing (NGS), or to ascertain relatives of individuals carrying variants that have already been identified, or to complement previous sequencing analyses that were unable to detect changes in the copy number of one or more exons in the genes under investigation.

MLPA is a semi-quantitative, nonautomated technique that can estimate the copy number of sixty or so sequences by multiplex PCR.

MLPA (Multiplex Ligation-dipendent Probe Amplification)

The principle of MLPA is based on the amplification of up to 60 probes per kit, each of which is specific for a given DNA sequence. The reaction results in a series of amplicons of specific length, ranging from 64 to 500 nt, separated by capillary electrophoresis.

After an initial denaturation of the sample DNA, probes are added that consist of two oligonucleotides that must hybridize with directly adjacent target sequences in order to be ligated into a single probe.

During the subsequent PCR reaction, all bound probes are amplified simultaneously using the same primer pair, resulting in a series of unique amplicons. One of the primers is labeled with a fluorochrome, allowing visualization of the amplification products after separation of the fragments by capillary electrophoresis. Separation of the fragments produces a sample-specific electropherogram.

The amplification conditions are semiquantitative so that the relative height of each individual peak, compared with the relative heights of probe peaks in various reference DNA samples, reflects the number of copies of the corresponding target sequence in a sample. The inclusion of reference samples in the same analysis is therefore essential. Elimination of one or more target sequences is visible as a relative decrease in peak height, while an increase in relative peak height reflects an increase in copy number.

The software used for analysis are Gene Marker v.3.0.1 (SoftGenetics) and Coffalyser.Net v. 220513.1739 (MRC-Holland).

The analytical protocol has a sensitivity of 99 percent and a specificity of 97 percent in identifying extensive deletions/duplications (Vorstman et al., Hum Mutat;27(8), 2006).

Commercially available SALSA MLPA kits provided by the company MRC-Holland (most CE-IVD-marked) with specific probes for genes of interest, The test is performed on genomic DNA (gDNA) obtained from the patient's blood sample or extracted from paraffin-embedded tumor tissue (FFPE).

Identified variants are always confirmed on second aliquot with alternative SALSA MLPA kits, or by different methods such as QPCR, Long Range PCR or NGS.

Limits of the analysis

MLPA cannot detect any deletions or duplications that lie outside the probes' target sequence and cannot detect balanced inversions or translocations.

In addition, variants under the probes or in close proximity can give false positives so this should always be checked if only one probe is lost.

In the case of poor denaturation of sample DNA, they can give false positives especially extremely GC-rich chromosome regions are not denatured at 98°C when more than 40 mM NaCl or KCl is present.

Optimal results are obtained by using good quality DNA, obtained from blood, or at least not too fragmented.

MRC Holland provides a sheet for each kit that describes the design of the probes and the purpose of the panel to be referred to for analysis

Conclusions

MLPA is a reliable, reproducible and fast method, thanks in part to the stability and efficiency of the commercial kits used and the accuracy and precision of the analysis software our laboratory has.