Multiplex PCR and Multiplex ligation-dependent probe amplification (MLPA)

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Multiplex PCR is a technique of Polymerase Chain Reaction (PCR) that brings the advantage of performing several amplifications of DNA in a single reaction. The DNA is amplified in samples using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler.

Since multiple reactions are done in one instance, several variables such as temperature, primers, enzymes and DNA target sequences have to be taken into consideration in order to avoid any interferences that may form between them, such as primer dimers or false negative results.

The primers and target sequences have to be chosen in such a way as to avoid the formation of dimers. Amplicons require similar annealing temperatures to avoid double-strand formation errors. They also require similar sizes to avoid nucleotide competition where the amplification of one amplicon uses most of the available nucleotides, lowering the amplification rate of the remaining amplicons and giving off a false positive result. The resulting problem of overlapping bands in an electrophoresis gel is circumvented with the use of fluorescent marker dyes. Buffer optimization is also required, usually needing more dNTPs, magnesium and polymerase when compared to the standard “singleplex” PCR.

The main advantages associated with multiplex PCR are the fact that it saves a lot of time and resources, such as DNA samples and reagents. It also decreases errors associated with repetition like pipetting errors and possible contaminations.

The main disadvantage of multiplex PCR stems from the many parameters that have to be taken into consideration: some combinations of DNA targets are very limiting to work with due to intrinsic design issues.

Multiplex PCR was referred for the first time in an article in 1988 by J. Chamberlain *et al* to diagnose a disease called “Duchenne muscular dystrophy” or DMD. This technique was used to detect deletions in the dystrophin gene in order to have a pre-natal or post-natal diagnosis of the referred disease.

Those affected by DMD have partial gene deletion on different locations. There are 6 specific exons that are deletion-sensitive. Any patient that is missing one or more of them is therefore diseased. Diagnosing DMD via multiplex PCR consists in amplifying these 6 exons simultaneously and verifying their existence (if amplification occurred or not). If this were done via “singleplex” PCR then each diagnosis would require six PCR reactions, one for each exon.

Over the years, different variations of this technique have been developed. We will emphasize Multiplex ligation-dependent probe amplification (MLPA).

MLPA is a semi-quantitative multiplex PCR generally used to detect changes in the number of copies in the chromosomal DNA in multiple targets, with the use of only one pair of primers. Its detection is very precise, being able to detect sequences with only 60 nucleotides and identify deletions and amplification in a single exon.

This technique needs the design of two adjacent probes that contain the forward and reverse primer sequence for the short sequence of target DNA. These probes contain a “stuffer sequence” of variable length.

The probes are hybridized into the target DNA and a functional PCR is performed. If the target DNA is present in the sample, a PCR strand appears, signaling both amplification and successful hybridization of the probes. Furthermore, the amount of PCR product is proportional to the amount of target DNA present in the sample, making this technique suitable for quantitative measurements.

Since each complete probe has a unique length, many amplicons can be separated and identified through electrophoresis, solving the resolution limitations of multiples PCR.

This allows many applications such as detection of mutations and single nucleotide polymorphisms, diagnose of cancer predisposition, neuromuscular disorders, intellectual disabilities and solid tumors, etc.

References

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