

Abstract

Detecting copy number changes in genomic DNA using multi-colour multiplex ligation-dependent probe amplification

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Genomic deletions and duplications play an important role in the etiology of human disease. Versatile tests are required to detect such rearrangements, and these tests should be readily applicable in diagnostic laboratories. Multiplex Ligation-dependent Probe Amplification (MLPA) is such a technique, allowing the rapid and precise quantitation of multiple sequences within a nucleic acid sample using a one-tube assay. MLPA is based on the hybridization of two directly flanking half-probes, their ligation and subsequent amplification by PCR. Common primer tails on mixed probes mean that all can be amplified with one primer pair. Chemically synthesizing two half-probes as oligonucleotides facilitates rapid probe development, but length restraints in oligo synthesis limit the number of probes that can be used simultaneously. We have partially circumvented this restriction by using different colours and capillary electrophoresis, i.e. we combine two or more probe sets in a single reaction, with each set using a specific pair of amplification primers. Each primer pair generates products labeled with a different fluorophore, with the primers selected such that all products can be amplified under the same reaction conditions. To increase the complexity further, we have started to analyse the products on a micro-array, obviating the need for probes of different lengths. Consequently, thousands of loci could be examined at resolutions far greater than that achieved with FISH or array CGH. Initial experiments show that, even using a mix containing >250 probes, single exon deletions can still be detected.

We have applied MLPA in a diagnostic setting for the analysis of several specific diseases, e.g. Duchenne muscular dystrophy (DMD) and multiple hereditary exostoses (EXT1 and EXT2), with excellent results. In one application, to identify trisomies, we were able to perform the assay, from blood sample to result, in less than 8 hours. We are currently expanding on previous work using MAPH to detect genomic deletions and duplications involved in mental retardation. Examining >200 samples at ~250 loci showed several rearrangements, not only in patients, but also in unaffected relatives and healthy controls.

The fact that virtually any combination of probes can be quickly synthesized and combined in a single reaction, coupled with the speed and flexibility of analysis afforded by different platforms means that this technique has an important role to play in molecular cytogenetics.