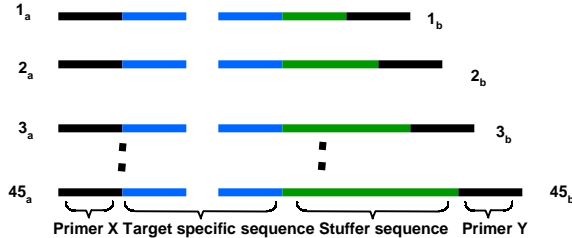


MLPA

Cheap and Sensitive Genomic Profiling MRC-Holland, Amsterdam

1. Probe Design



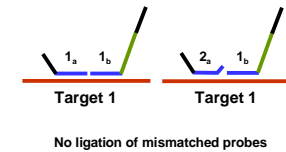
Multiplex Ligation-dependent Probe Amplification (MLPA) is a new method to detect an unusual copy number of up to 45 different sequences in a single, simple to perform, reaction. In MLPA added probes rather than the sample nucleic acid are amplified in a multiplex PCR using only one PCR primer pair. Amplification products are separated by sequence type electrophoresis, resulting in a very reproducible gel pattern. Comparing the sample pattern with a control sample will indicate aberrant copy numbers of the target sequences.

Performing an MLPA reaction requires a thermocycler with heated lid and sequence type capillary electrophoresis equipment. MLPA is able to discriminate between sequences that differ in only one nucleotide and requires a minimum of only 20 ng human DNA or 5 ng of total RNA.

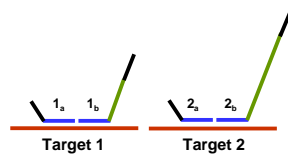
MLPA has been described by Schouten et al: Relative quantification of 40 nucleic acid sequences by multiplex ligation dependent probe amplification. *Nucleic Acid Research* (2002) 30, e57.

MLPA kits are available from MRC-Holland, Amsterdam, the Netherlands

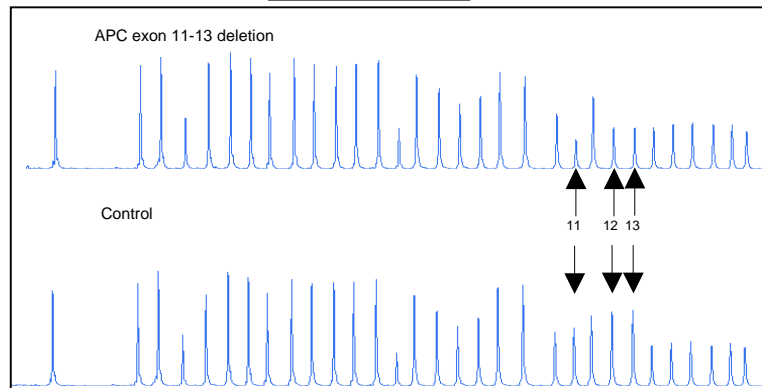
3. Ligation



2. Multiplex Hybridization



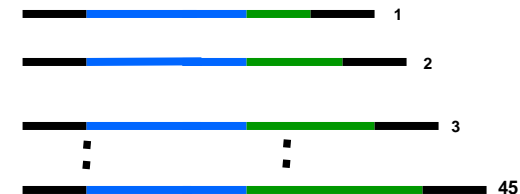
5. Fragment Analysis



Detection of APC exon 11-13 deletion. Samples containing approximately 100 ng of DNA were analysed by MLPA using probe mix P043. Control DNA was obtained from Promega. Blood-derived DNA from an individual known to contain a deletion of exons 11-13 was provided by the Swedish Adenomatous Polyposis Registry, Stockholm, Sweden. Reactions were analysed by capillary electrophoresis (ABI 310). Probe mix P043 contains 31 probes. Probes are present for each of the 15 APC exons and 11 control probes that recognise other sequences on various chromosomes.

Exon deletion are apparent by an ~50% reduction in peak area of a specific probe. More information on the P043 MLPA kit can be obtained at www.mrc-holland.com.

4. PCR with universal primers X and Y



Only ligated probes are exponentially amplified