



## Improved detection of copy number variation and SNPs

- This technology allows for the efficient and reliable detection of Copy Number Variation (CNV) and multiple SNPs in one test.
- This technology reduces the cost of SNP and copy number variation detection by allowing for the use of short synthetic probes.
- This technology increases the specificity of MLPA allowing for the testing of CNV in highly variable regions of the genome such as KIR genes (Killer-cell immunoglobulin-like receptors) involved in many immune responses

Diagnostics | Copy number variation/SNP

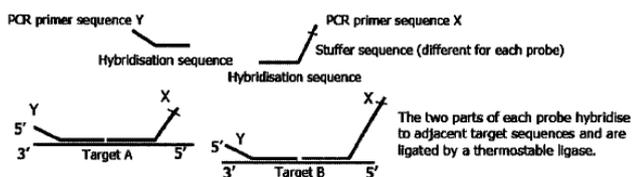
2011

### Background

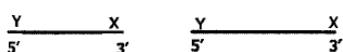
Multiplex Ligation-dependent Probe Amplification (MLPA) is a relatively new multiplex PCR method detecting abnormal copy numbers of different genomic DNA- or RNA sequences, which is able to distinguish sequences differing in only one nucleotide.

The unique combination of effective CNV detection and the ability to detect single nucleotide polymorphisms (SNPs) make MLPA a powerful tool to screen for genetic disorders, such as Duchenne, DiGeorge syndrome, SMA, hereditary pancreatitis, Anti-thrombin deficiency, Birt-Hogg-Dube syndrome.

Although the technique has many advantages over PCR, DHPLC, FISH and CGH it currently cannot detect more than 1 SNP per probe set and if SNPs are located too close to each other probe sets can interfere with each other.



All probe ligation products are amplified by PCR using only one primer pair.



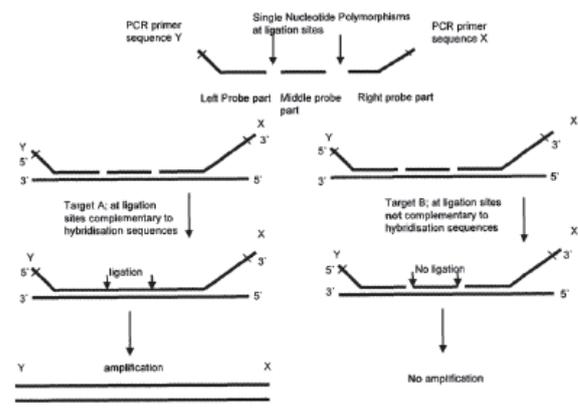
Amplification products are separated by electrophoresis. Relative amounts of probe amplification products, as compared to a control DNA sample, reflect the relative copy number of target sequences.

### The Technology

Researchers at Sanquin have developed an improved MLPA method based on 3 primer sequences, in contrast to the standard 2 primers. The use of a third primer sequence has a number of benefits:

- it allows for the detection of 2 SNPs, instead of only one
- it allows for the use of shorter primer sequences allowing for the use of significantly cheaper, short length synthetic primers
- The technique is more sensitive and can detect SNPs up to 20 nt apart whilst retaining its specificity.

These properties combined with MLPA's ability to detect CNV accurately make this technique ideal for investigating certain variable regions of the genome such as the KIR genes. CNV of KIR genes are reported to play an important role in auto-inflammatory reactions, hematopoietic stem cell transplantation (leukemia), progression of AIDS, HCV and CMV



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## Intellectual Property

Patent nr. US12/998,595

[Direct link.](#)

## Inventors

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- Prof TW Kuijpers MD PhD (also Professor in Pediatric Immunology, AMC)  
- M. de Boer, Ing

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## Key publications

1. Breunis WB, van Mirre E, Bruin M, Geissler J, de Boer M, Peters M, Roos D, de Haas M., Koene HR, Kuijpers TW. Copy number variation of the activating FCGR2C gene predisposes to idiopathic thrombocytopenic purpura. *Blood* 2008; 111(3): 1029-1038.
2. Breunis WB, van Mirre E, Geissler J, Laddach N, Wolbink GJ, van der Schoot E, de Haas M, de Boer M, Roos D, Kuijpers TW. Copy number variation at the FCGR locus includes FCGR3A, FCGR2C and FCGR3B but not FCGR2A and FCGR2B. *Hum Mutat* 2009; 30(5): E640-50.