SALSA® MLPA®

The gold standard for DNA copy number quantification

Coffalyser.Net: MLPA® analysis software

Free MLPA data analysis software designed and supported by MRC Holland.

- User-friendly software and reliable MLPA data analysis
- Extensive quality control developed specifically for MLPA
- Immediate access to the latest analysis panels (Coffalyser sheets)
- Server-client model that allows data sharing
- Available free of charge!

Collaborations with scientists

Most novel MLPA applications are developed in close collaboration with scientists around the world. Results obtained with MLPA probemixes have been described in thousands of scientific publications. Researchers are encouraged to contact us with requests for new MLPA applications or feedback on current panels on info@mrcholland.com.

MRC Holland

Willem Schoutenstraat 1
1057 DL Amsterdam
the Netherlands

tel +31 88 865 7200
info@mrcholland.com
www.mrcholland.com

GB-M-G-011D

MLPA®

Multiplex Ligation-dependent Probe Amplification (MLPA) is a multiplex PCR-based method that can detect the copy number of up to 60 DNA sequences in a single reaction. 96 DNA samples can be handled simultaneously, with results being available within 24 hours.

In addition to copy number changes, MLPA allows for the detection of select known point mutations. Furthermore, MLPA is able to detect methylation patterns in DNA when used in combination with a methylation-sensitive restriction enzyme (MS-MLPA). MLPA is used worldwide for diagnostics and research of human genetic disorders and tumours.

Simultaneous detection

of copy number, methylation and select known point mutations.

Low input

Requires only 50 ng of DNA.

Time-efficient

Results available within 24 hours.

Short hands-on time

MLPA is performed in 5 simple steps.

Cost-effective

One MLPA reaction costs EUR 12/USD 15.

MLPA® Protocol

1. Sample DNA denaturation
- Sample DNA is heated to fully denature the DNA

2. Hybridisation of probes to sample DNA
- SALSA MLPA Buffer and a SALSA MLPA Probemix consisting of up to 60 probes are added to the sample

3. Ligation of hybridised probes
- Hybridised probes are ligated by adding SALSA Ligase-65 enzyme and SALSA Ligase Buffers to form fully amplifiable probes

4. PCR amplification of ligated probes
- Ligated MLPA probes are amplified by adding SALSA Polymerase and a single fluorescently-labelled primer pair

5. Fragment separation by capillary electrophoresis
- MLPA PCR products are directly loaded onto a CE device

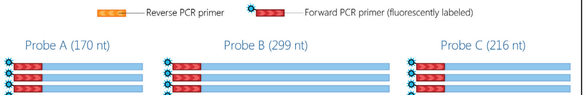
6. Analysis by Coffalyser.Net

How MLPA® works

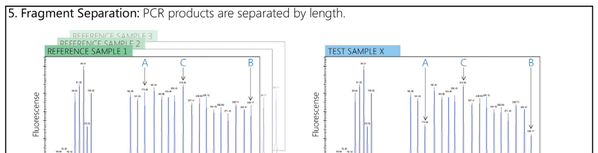
1. Denaturation/2. Hybridisation: Left (LPO) and Right Probe Oligo (RPO) bind to their target DNA.

3. Ligation: Hybridised probe oligos are ligated by ligase enzyme.

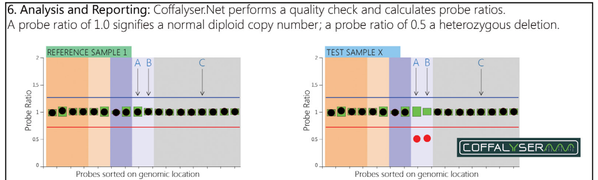
4. Amplification: Ligated probes are amplified using a single primer pair.



5. Fragment Separation: PCR products are separated by length.



6. Analysis and Reporting: Coffalyser.Net performs a quality check and calculates probe ratios.
A probe ratio of 1.0 signifies a normal diploid copy number; a probe ratio of 0.5 a heterozygous deletion.



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MLPA® Applications

MRC Holland offers over 350 MLPA assays for copy number detection. Visit our website www.mrcholland.com to find the assay for your genes and applications of interest.

Predisposition to Cancer
- Breast Cancer (*BRCA1/2, CHEK1/2, TP53*)
- Lynch Syndrome (*MLH1*, MSH2/6*, PMS2**)
- Neurofibromatosis (*NF1/2*)
- *PTEN, STK11, CDH1, PALB2, ATM*

Metabolic & Mitochondrial Disorders
- *LDLR*
- *GLA*
- *CYP450*
- Wilson's Disease

Neuromuscular Disorders
- Spinal Muscular Atrophy (*SMN1, SMN2*)
- Duchenne Muscular Dystrophy (*DMD*)
- Charcot-Marie-Tooth Disease
- Limb Girdle Muscular Dystrophy

Neurological Disorders
- Parkinson's Disease
- Hereditary Spastic Paraplegia
- Epilepsy (*KCNQ2/3, SCN1A*)
- Dopa-responsive Dystonia

Hereditary Blood Disorders
- Thalassemia (Alpha, Beta)
- Fanconi Anemia
- Clotting Factor Deficiencies (V, IX, X, XI)
- Von Willebrand Disease

Skeletal & Connective Tissue
- Ehlers-Danlos (*PLOD1, COL3A1/5A1*)
- Marfan Syndrome
- Osteogenesis Imperfecta (*COL1A1/2, PLS3*)
- *SHOX*

Lung Disorders
- Cystic Fibrosis
- Primary Ciliary Dyskinesia
- Alveolar Capillary Dysplasia
- AAT-deficiency

Tumour Profiling
- Tumour suppressors: *IKZF1, TP53, RB1**
- Blood cancers: *ALL, MDS, CLL, MM*
- Breast: *ERBB2, CDH1, CCNE1*, BRCA1ness
- Glioma: 1p, 19q, *IDH1, IDH2, MGMT**

Intellectual Disability
- Prader Willi/Angelman Syndrome*
- Subtelomeric Testing
- Microdeletion Syndromes
- Tuberous Sclerosis, Rett, DiGeorge, UPD7/14*

Cardiovascular Disorders
- Marfan Syndrome
- HHT/HPAH
- Loeys-Dietz Syndrome
- Familial Hypertrophic Cardiomyopathy

Endocrinological Disorders
- Congenital Adrenal Hyperplasia
- MODY
- Multiple Endocrine Neoplasia (*MEN1*)
- Albright Hereditary Osteodystrophy (*GNAS*)*

Kidney Disorders
- Alport Syndrome
- ADPKD
- ARPKD
- Birt-Hogg-Dube Syndrome

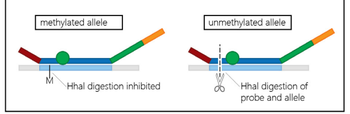
MLPA probemixes are for Research Use Only. Not for Use in Diagnostic Procedures unless explicitly stated otherwise.
* For this gene/application, both copy number and DNA methylation can be determined.

MS-MLPA®

Methylation-Specific MLPA (MS-MLPA) combines MLPA with the use of the methylation-sensitive endonuclease HhaI, allowing the simultaneous detection of both DNA copy number and methylation status, with no bisulfite treatment required.

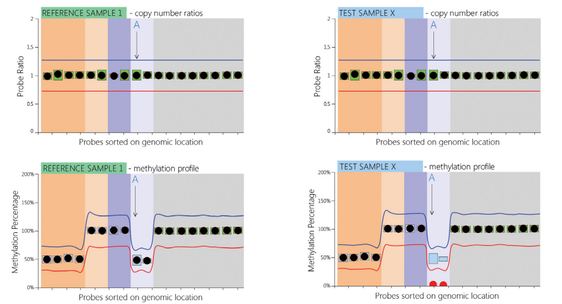
3A. Ligation: Copy Number Analysis

3B. Ligation and HhaI Digestion: Methylation Analysis



In MS-MLPA, the hybridisation reaction is split in two: a normal MLPA reaction is performed to quantify DNA copy numbers present in the sample (see 3A) and a simultaneous ligation and digestion reaction is performed (see 3B). Methylation of the target DNA protects the probe-sample DNA hybrid strand against digestion by HhaI (see 3B, left). In contrast, probes bound to unmethylated DNA targets will be digested by the enzyme, and will not produce a probe signal (see 3B, right).

6. Analysis & Reporting (by COFFALYSER.NET)



Results: the copy number ratios of test sample X (top right) are compared to those in the reference samples (one shown, top left). Test sample X shows no aberration in copy numbers. Subsequently, the methylation pattern of the test sample (bottom right) can be compared to that of the reference samples (one shown, bottom left). Test sample X shows an epigenetic abnormality: two DNA probe targets that are 50% methylated in unaffected individuals (blue boxes), are completely unmethylated in test sample X (red dots).