

MLPA®

General information

GB-M-G-010D

Coffalyser.Net™



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@mlpa.com.



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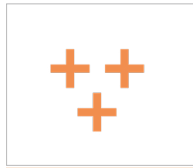
MRC-Holland
MLPA®

MLPA[®]

The gold standard in copy number quantification

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.



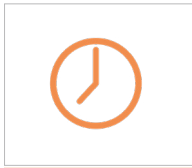
Simultaneous detection

of copy number, methylation and select known point mutations.



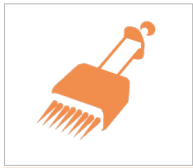
Low input

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. DNA denaturation

- Incubate 5 µl DNA sample for 5 minutes at 98°C

2. Hybridisation of probes to sample DNA

- Cool down to room temperature, open tubes
- Add 3 µl Hybridisation master mix
- Incubate 1 minute at 95°C + 16 hours at 60°C

3. Ligation of hybridised probes

- Lower thermocycler temperature to 54°C, open tubes
- Add 32 µl Ligase-65 master mix, incubate 15 minutes at 54°C
- Heat inactivate the ligase enzyme: 5 minutes at 98°C

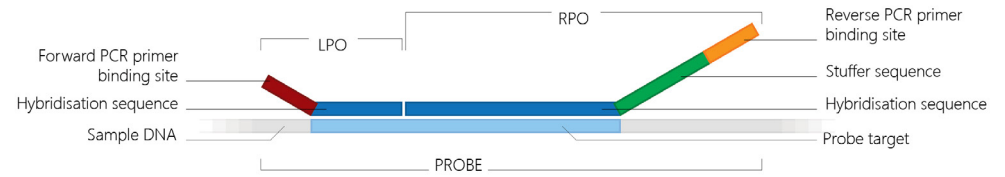
4. PCR amplification of ligated probes

- Cool down to room temperature, open tubes
- Add 10 µl Polymerase master mix at room temperature
- Start PCR

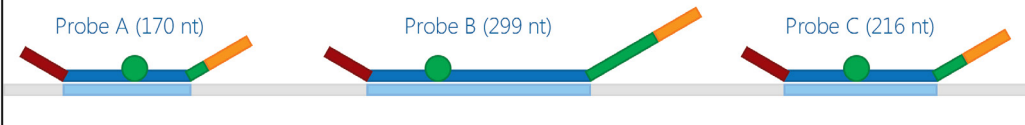
5. Fragment separation by capillary electrophoresis

How MLPA[®] works

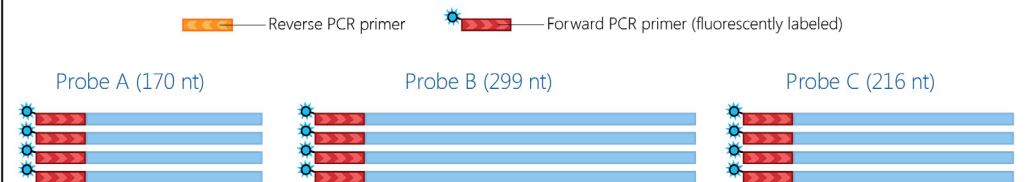
1. Denaturation/2. Hybridisation: Left (LPO) and Right Probe Oligo (RPO) bind to their target DNA.



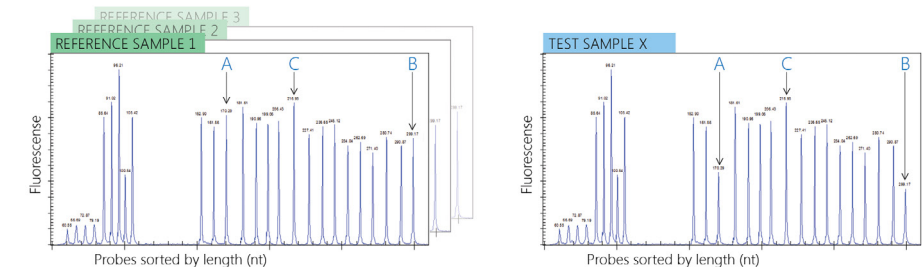
3. Ligation: Hybridised probe oligos are ligated by ligase enzyme.



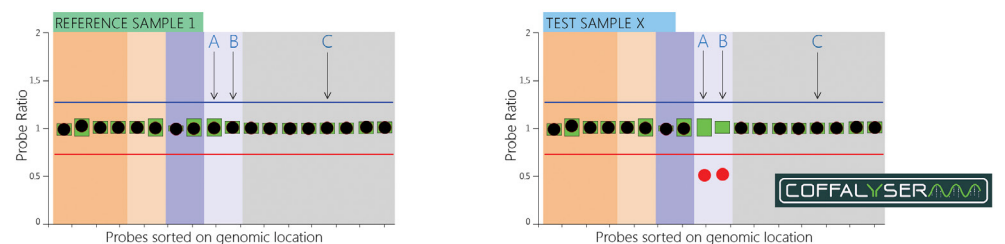
4. Amplification: Ligated probes are amplified using a single primer pair.



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







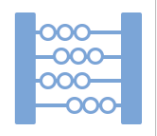
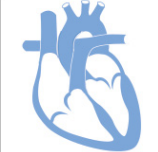
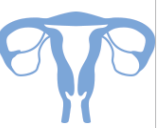
6. Analysis and Reporting: Coffalyser.Net performs a quality check and calculates probe ratios. A probe ratio of 1.0 signifies a normal diploid copy number; a probe ratio of 0.5 a heterozygous deletion.



MLPA® Applications

Select popular MLPA probemix applications

MRC-Holland offers over 400 MLPA assays for copy number detection. Visit www.mlpa.com to find the assay for your genes and applications of interest.

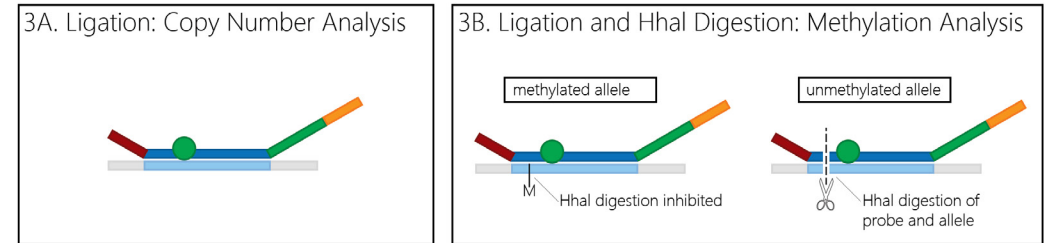
 <p>Predisposition to Cancer</p> <ul style="list-style-type: none"> Breast Cancer (BRCA1/2, CHEK1/2, TP53) Lynch Syndrome (MLH1*, MSH2/6*, PMS2*) Neurofibromatosis (NF1/2) PTEN, STK11, CDH1, PALB2, ATM 	 <p>Metabolic & Mitochondrial Disorders</p> <ul style="list-style-type: none"> LDLR GLA CYP450 Wilson's Disease
 <p>Neuromuscular Disorders</p> <ul style="list-style-type: none"> Spinal Muscular Atrophy (SMN1, SMN2) Duchenne Muscular Dystrophy (DMD) Charcot-Marie-Tooth Disease Limb Girdle Muscular Dystrophy 	 <p>Neurological Disorders</p> <ul style="list-style-type: none"> Parkinson's Disease Hereditary Spastic Paraplegia Epilepsy (KCNQ2/3, SCN1A) Dopa-responsive Dystonia
 <p>Hereditary Blood Disorders</p> <ul style="list-style-type: none"> Thalassemia (Alpha, Beta) Fanconi Anemia Clotting Factor Deficiencies (V, IX, X, XI) Von Willebrand Disease 	 <p>Skeletal & Connective Tissue</p> <ul style="list-style-type: none"> Ehlers-Danlos (PLOD1, COL3A1/5A1) Marfan Syndrome Osteogenesis Imperfecta (COL1A1/2, PLS3) SHOX
 <p>Lung Disorders</p> <ul style="list-style-type: none"> Cystic Fibrosis Primary Ciliary Dyskinesia Alveolar Capillary Dysplasia AAT-deficiency 	 <p>Tumour Profiling</p> <ul style="list-style-type: none"> Tumour suppressors: IKZF1, TP53, RB1* Blood cancers: ALL, MDS, CLL, MM Breast: ERBB2, CDH1, CCNE1, BRCA1ness Glioma: 1p, 19q, IDH1, IDH2, MGMT*
 <p>Intellectual Disability</p> <ul style="list-style-type: none"> Prader Willi/Angelman Syndrome* Subtelomeric Testing Microdeletion Syndromes Tuberous Sclerosis, Rett, DiGeorge, UPD7/14* 	 <p>Cardiovascular Disorders</p> <ul style="list-style-type: none"> Marfan Syndrome HHT/HPAH Loeys-Dietz Syndrome Familial Hypertrophic Cardiomyopathy
 <p>Endocrinological Disorders</p> <ul style="list-style-type: none"> Congenital Adrenal Hyperplasia MODY Multiple Endocrine Neoplasia (MEN1) Albright Hereditary Osteodystrophy (GNAS)* 	 <p>Kidney Disorders</p> <ul style="list-style-type: none"> 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.

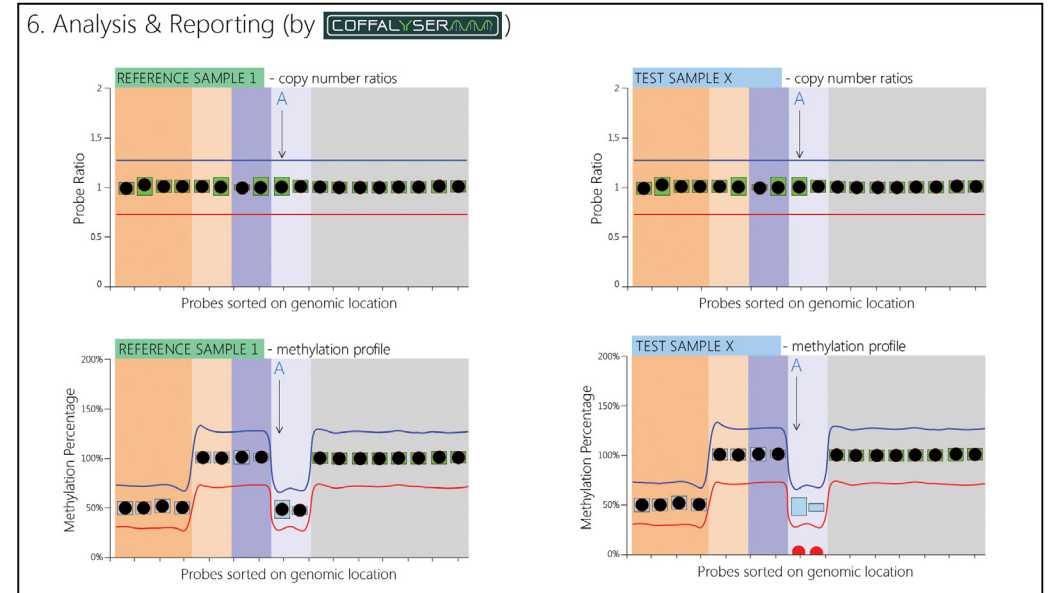
MS-MLPA®

Easy, reliable and bisulfite-free methylation analysis

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.



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).



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).