SALSA® MLPA®

Predisposition to Cancer

MRC Holland

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.

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MLPA® & Predisposition to Cancer

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

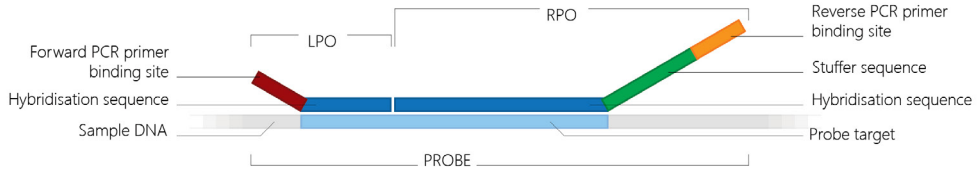
4. PCR amplification of ligated probes
- Ligated MLPA probes are amplified by adding SALSA Polymerase and a single fluorescently-labelled primer pair

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

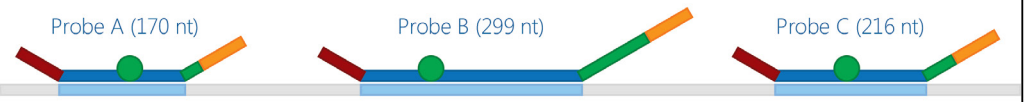
6. Analysis by Coffalyser.Net

How MLPA® works

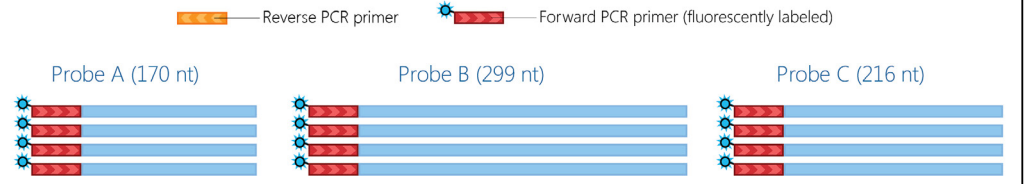
1. Denaturation/2. Hybridisation: Left (LPO) and Right Probe Oligo (RPO) bind to their target DNA.



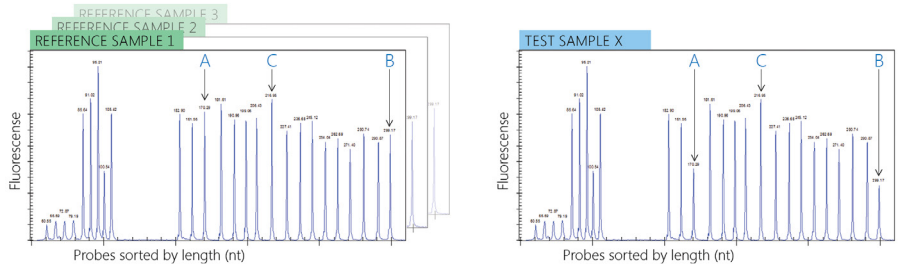
3. Ligation: Hybridised probe oligos are ligated by ligase enzyme.



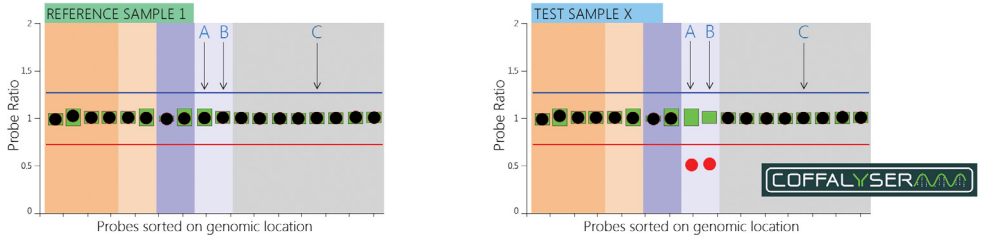
4. Amplification: Ligated probes are amplified using a single primer pair.



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.



MLPA® Probemixes: Predisposition to Cancer

Over 350 MLPA probemixes are available and new assays are continuously developed in close collaboration with scientists around the world. The following list gives an overview of current MLPA probemixes for predisposition to cancer. See www.mrcholland.com for a complete overview.

Application	Probemix	Main target genes
Ataxia Telangiectasia	P041-P042	*ATM*
Cowden Syndrome	P225	*PTEN*
DICER1 Syndrome and Pleuro-pulmonary Blastoma	P482	*DICER1*
Familial Hyperparathyroidism + HPT Jaw Tumour Syndrome	P466	*CDC73*
Familial MDS-AML	P437	*GATA2* (+R398W, T354M), *TERC, TERT, CEBPA, RUNX1*
Familial Medulloblastoma and Meningioma	P472	*SUFU*
Familial Melanoma	P419	*CDKN2A/2B, CDK4, MITF* E318K
	ME024	*CDKN2A/2B**, flanking regions
Familial Meningioma	P478	*SMARCE1*
Fanconi Anemia	P031-P032	*FANCA*
	P057	*FANCD2, PALB2*
	P113	*FANCB*
	P260	*PALB2, RAD50, RAD51C, RAD51D*
Gorlin Syndrome	P067	*PTCH1*
Hereditary Breast & Ovarian Cancer	P002	*BRCA1* Primary test | P087 Confirmatory
	P045	*BRCA2, CHEK2* Primary test | P077 Confirmatory
	P090	*BRCA2* Primary test | P077 Confirmatory
	P239	*BRCA1* region
Breast Cancer, Increased Susceptibility To	P041-P042	*ATM*
	P057	*FANCD2, PALB2*
	P190	*CHEK2, ATM, TP53*
	P240	*BRIP1, CHEK1*
	P260	*PALB2, RAD50, RAD51D, RAD51C*
Hereditary Diffuse Gastric Cancer	P083	*CDH1*
Juvenile Polyposis Syndrome	P158	*BMPR1A, SMAD4, PTEN*
Li-Fraumeni Syndrome	P056	*TP53, CHEK2* 1100delC

Application	Probemix	Main target genes
Lynch Syndrome	P003	*MLH1, MSH2* Primary test | P248 Confirmatory
	P008	*PMS2*
	P072	*MSH6, MUTYH, MSH2, EPCAM*
	ME011	*EPCAM, BRAF* V600E + Methylation profiling of *MLH1, MSH2, MSH6, PMS2*
Melanocytic Tumours, Mesothelioma	P417	*BAP1*
Multiple Endocrine Neoplasia	P017	*MEN1*
	P244	*AIP, MEN1, CDKN1B*
Multiple Osteochondromas	P215	*EXT1, EXT2*
Neurofibromatosis	P081-P082	*NF1*
	P044	*NF2*
	P122	*NF1*-area (*17q11*)
	P295	*SPRED1*
Papillary Renal Carcinoma	P308	*MET, PTEN, LRRK2*
Paragangliomas and Pheochromocytoma	P226	*SDHB, SDHC, SDHD, SDHAF1, SDHAF2*
	P429	*SDHA, MAX*
Peutz-Jeghers Syndrome	P101	*STK11*
Polyposis Syndrome	P043	*APC*
	P378	*MUTYH, GREM1, SCG5*
Retinoblastoma	P047	*RB1**
Rhabdoid Predisposition Syndrome + Schwannomatosis	P258	*SMARCB1*
	P455	*LZTR1*
Tuberous Sclerosis	P046	*TSC2* Primary test | P337 Confirmatory
	P124	*TSC1*
Von Hippel-Lindau Syndrome	P016	*VHL*
Wilms' tumour, WAGR, Denys-Drash, Frasier Syndrome	P118	*WT1*

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.