MLPA®

Predisposition to Cancer

MRC-Holland MLPA®

SB-M-PtC-005D

ATM PTEN BRCA1 BRCA2 CHEK1
APC MSH2 PALB2 BRIP1 CHEK2
FANCA STK11 RAD50 MLH1 NF1
NF2 VHL SPRED1 TP53 MUTYH RB1

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® & 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. 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

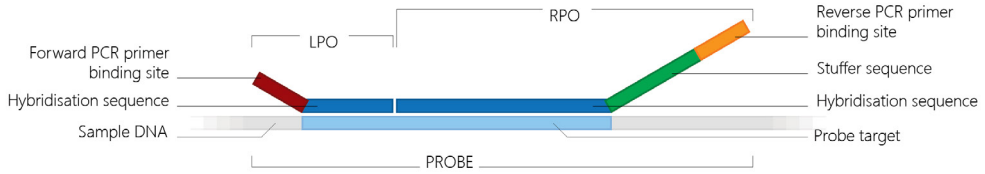
3. Ligation of hybridised probes
   - Lower thermocycler temperature to 54°C, open tubes
   - Add 32 µl Ligase-65 master mix, incubate 15 minutes at 54°C
   - Heat inactivate the ligase enzyme: 5 minutes at 98°C

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

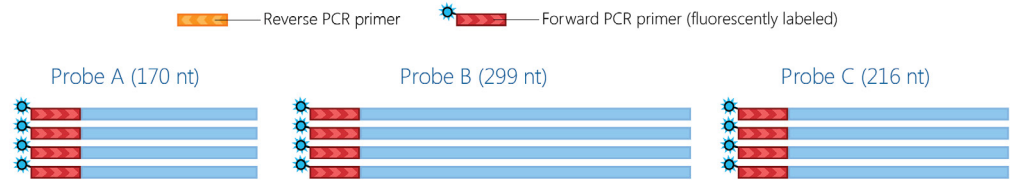
How MLPA® works

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

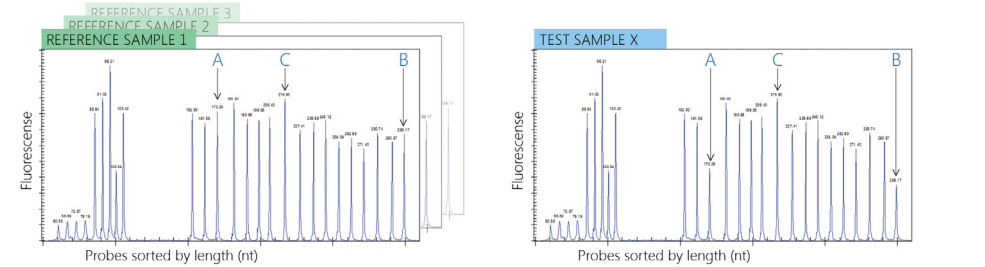


3. Ligation: Hybridised probe oligos are ligated by ligase enzyme.

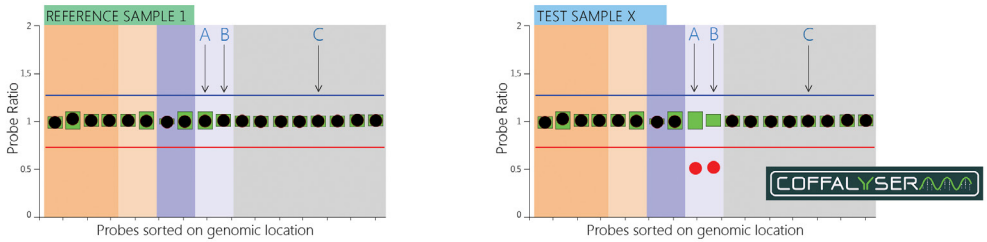
4. Amplification: Ligated probes are amplified using a single primer pair.



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.



D-M-0058

Probemixes: Predisposition to Cancer

Over 400 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.mlpa.com for a complete overview.

Application	Probemix	Main target genes		
Ataxia Telangiectasia	P041-P042	ATM		
Cowden Syndrome	P225	PTEN		
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/B, CDK4, MITF E318K		
	ME024	CDKN2A/B*, 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		
	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		
Lynch Syndrome	P003	MLH1, MSH2	Primary test	| P248 Confirmatory
	P008	PMS2		
	P072	MSH6, MUTYH, MSH2, EPCAM		
	ME011	EPCAM + Methylation profiling of MLH1, MSH2, MSH6, PMS2		

Application	Probemix	Main target genes		
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.