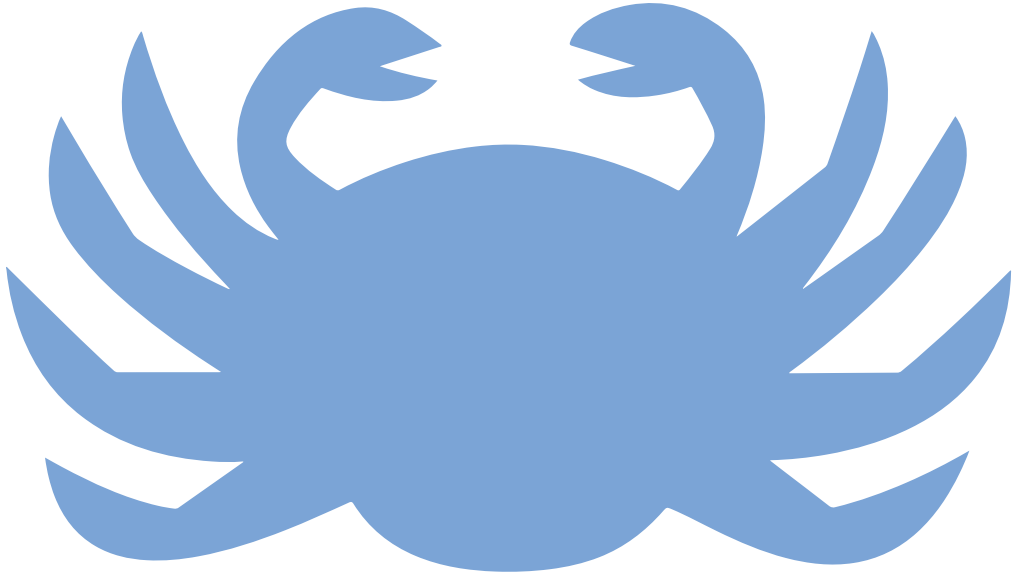
MLPA®

Tumour Profiling

MRC-Holland MLPA®

SB-M-TP-006D



RB1 PTEN 9p21 BAP1 FGFR1
ERG CCND1 MGMT MLH1 TP53
EGFR ERBB2 IKZF1 PIK3CA MYC
MYCN BRAF V600E JAK2 V617F

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® & Tumour Profiling

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. Suitable for DNA from FFPE tissue.

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

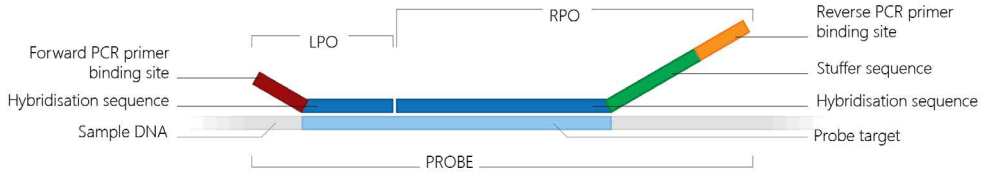
3. Ligation of hybridised probes
   - Lower thermocycler temperature to 54°C, open tubes
   - Add 32 µl Ligase-65 master mix, incubate 15 minutes at 54°C
   - Heat inactivate the ligase enzyme: 5 minutes at 98°C

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

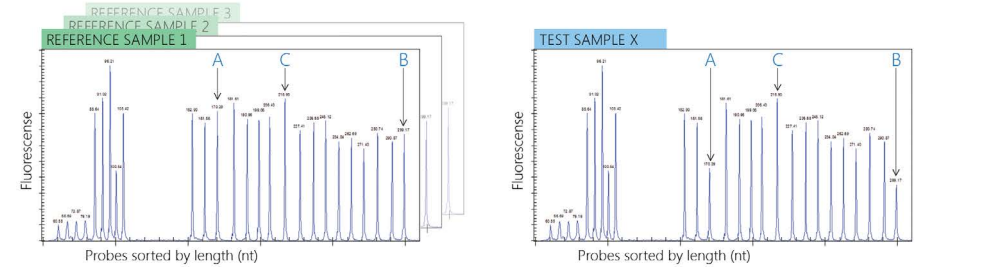
1. Denaturation/2. Hybridisation: Left (LPO) and Right Probe Oligo (RPO) bind to their target DNA.



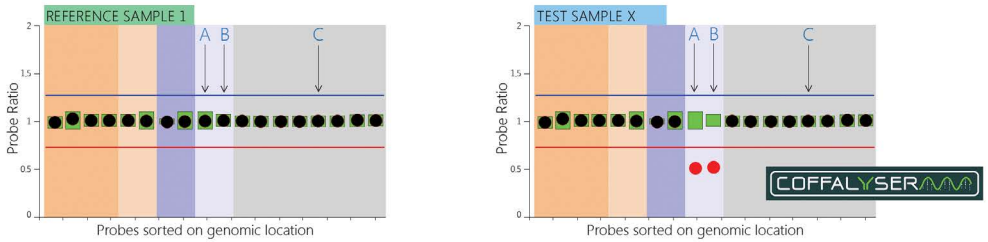
3. Ligation: Hybridised probe oligos are ligated by ligase enzyme.

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.



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Probemixes: Tumour Profiling

Over 400 MLPA probemixes are available and new assays are continuously developed in close collaboration with scientists around the world. The following lists give an overview of current MLPA probemixes for various applications in characterisation of tumour DNA. See www.mlpa.com for a complete overview.

Blood Cancers

Application	Probemix	Genes/region
Acute Lymphoblastic Leukaemia (ALL)	P202	IKZF1, ERG, CDKN2A/2B, 14q32
	P327	iAMP21, RUNX1, ERG
	P329	Xp22.33 PAR1 region (SHOX, CRLF2, CSF2RA, IL3RA)
	P335	IKZF1, PAX5, ETV6, RB1, BTG1, EBF1, 9p21.3 (CDKN2A/B), Xp22.33 PAR1 region
	P383	STIL-TAL1, LEF1, CASP8AP2, MYB, EZH2, CDKN2A/B, MTAP, MLLT3, NUP214-ABL1, PTEN, LMO1, LMO2, NF1, SUZ12, PTPN2, PHF6
	ME024	CDKN2A/B*, MTAP, MIR31*, CDKN2B-AS1*, PAX5
Chronic Lymphoblastic Leukaemia (CLL)	P037	11q22.3 (ATM), chr. 12, 13q14, 17p13 (TP53), 2p, 6q, 8p/q, 9p21
	P038	11q22-q23, chr. 12, 13q14, 17p13 (TP53), 10q23, 14q32, chr. 19, NOTCH1 7541_7542delCT, SF3B1 K700E, MYD88 L265P point mutations
	P040	11q13-q25, chr. 12, 13q14, 17p13 (TP53)
Follicular Lymphoma	P462	1p (TNFRSF14), 1q, 2p (REL), 3q (BCL6), 6q (EPHA7, PRDM1, TNFAIP3), 7q (EZH2), 8q (MYC), 9p (CDKN2A/B), 10q (PTEN, FAS), 12q, 15q (B2M), 17p (TP53), 18q (MALT1, BCL2), Xp11 (BCOR, KDM6A)
Hematologic Malignancies	P377	2p (MYCN, ALK), 5q (MIR145, EBF1, MIR146A), 6q, 7p12 (IKZF1), 7q, 8q24 (MYC), 9p (JAK2 V617F mutation, MTAP, CDKN2A/B, PAX5), 10q23 (PTEN), 11q22.3 (ATM), 12p (ETV6), 12q, 13q (RB1, MIR15A, DLEU1/2), 17p (TP53), 17q, chr. 18, chr. 19, 21q (RUNX1)
Myelodysplastic Syndromes (MDS)	P414	Chr. 3, 5q, 7q (EZH2), 8q (MYC), 11q (KMT2A), 12p (ETV6), chr. 17 (TP53, NF1, SUZ12), chr. 19, 20q, chr. Y, JAK2 V617F point mutation
Myeloproliferative Neoplasms (MPNs)	P520	Point mutation detection with only >1 % mutation burden for JAK2 (V617F, E543_D544del, N542_E543del), CALR (52-bp deletion, 5-bp insertion), MPL (W515L, W51K), KIT (D816V)
	P420	Point mutation detection with only >10 % mutation burden for JAK2 (V617F, E543_D544del, N542_E543del), CALR (52-bp deletion, 5-bp insertion), MPL (W515L, W51K), KIT (D816V)
Multiple Myeloma	P425	1p32-p12, 1q21-q23, 5q31, chr. 9, 12p13, 13q14 (RB1, DLEU1/2), 14q32 (TRAF3), 16q12-q23 (CYLD, WWOX), 17p13 (TP53)

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 using methylation-specific MLPA (MS-MLPA). MS-MLPA is a variant of the MLPA technique in which copy number detection is combined with the use of a methylation-sensitive restriction enzyme, for semiquantative detection of methylation status. The MS-MLPA protocol is very similar to the conventional MLPA method, except that the samples are split after the hybridisation step, generating two samples: one for copy number detection and one for methylation detection.

General Tumour Profiling

Genes/region	Probemix
ARID1A, ARID1B	P433
BAP1	P417
BRAF, HRAS, KRAS, NRAS	P298
CD274 (PD-L1)	P474
CDC73	P466
CDK4	P419
CDKN2A, CDKN2B	P419, ME024*
Centromeres	P181, P182
Chr. 8	P014
Chr. 16	P451

Genes/region	Probemix
Chr. 20q	P157
EGFR	P315
EXT1, EXT2	P215
FHIT	P063
JAK2	P474
LZTR1	P455
MAX	P429
MITF E318K	P419
multiple genes/regions	P175, P294, ME001*, ME002*

Genes/region	Probemix
PDCD1LG2 (PD-L2)	P474
PTEN	P225
RB1	P047*
SDHA	P429
SMARCB1	P258
SMARCE1	P478
SUFU	P472
TP53	P056
WWOX	P063
ZNRF3	P476

Solid Tumours

Application	Probemix	Genes/region
Breast Cancer	P078	6q (ESR1), 7p (EGFR), 8p (ZNF703, FGFR1, IKBKB)/8q (MTDH, MYC), 11q13 (CCND1, EMSY), 16q (CDH1), 17q12-q25 (ERBB2, TOP2A, BIRC5), 19q (CCNE1), 20q (AURKA)
	P376	BRCA1ness profile: 3p/q, 5q, 6p, 10p/q, 12p/q, 13q, 14q, 15q
Colon Cancer	ME011	EPCAM + Methylation profiling of MLH1, MSH2, MSH6, PMS2
	ME042	CpG Island Methylator Phenotype (CIMP)*
Glioma	P088	1p/q, 9p21, 19p/q, IDH1 R132H/C and IDH2 R172K/M point mutations
	P105	EGFR, PTEN, CDKN2A, TP53, PDGFRA, NFKBIA, CDK4, MIR26A2, MDM2
	P370	3p (SRGAP3-RAF1), 6q (MYB), 7q (KIAA1549-BRAF), 8p (FGFR1-TACC1), 8q (MYBL1), 9p21, IDH1 R132H/C, IDH2 R172K/M and BRAF V600E point mutations
	ME012	MGMT*, IDH1 R132H/C and IDH2 R172K/M point mutations
Gastric Cancer	P458	CCND1, CCNE1, CDK6, EGFR, ERBB2, FGFR1, FGFR2, GATA4, GATA6, KLF5, KRAS, MET, MYC, PIK3CA, PTP4A3, TOP2A
Medulloblastoma	P301-P303	Chr. 1, 2, 3, 4q, 5q, 6, 7, 8, 9, 10, 14q, 16, 17, 20
Neuroblastoma	P251-P253	Chr. 1, 2 (NBAS, DDX1, MYCN, ALK, BMPR2), 3, 4, 7, 9 (PTPRD, CDKN2A), 11, 12, 14q, 17 (TP53, WSB1)
Papillary Renal-Cell Carcinoma	P308	MET, PTEN, LRRK2
Sarcoma	P323	12p, 12q (GLI1, CDK4, HMGA2, MDM2)
Uveal Melanoma	P027	1p, chr. 3, chr. 6, chr. 8
Wilms' Tumour	P380	1p/q, 2p (MYCN), 2q, 4q (FBXW7), 11p (WT1), 16p/q, 17p (TP53), Xq11 (AMER1)