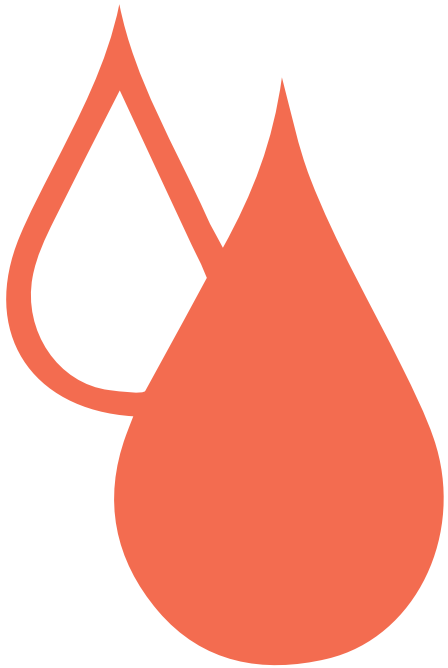
MLPA®

Hematologic Disorders

MRC-Holland MLPA®

SB-M-HD-090D



IKZF1 9p21 Fanconi Anemia ERG
TP53 NF1 EZH2 ALL CLL PROS1
VWF F8 HBA HBB JAK2 V617F
MDS MPL CALR GATA2 RUNX1

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® & Hematologic Disorders

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

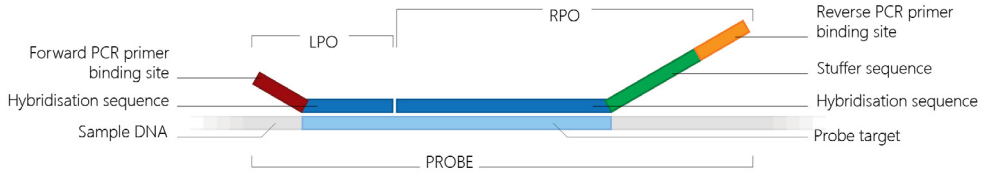
3. Ligation of hybridised probes
   - Lower thermocycler temperature to 54°C, open tubes
   - Add 32 µl Ligase-65 master mix, incubate 15 minutes at 54°C
   - Heat inactivate the ligase enzyme: 5 minutes at 98°C

4. PCR amplification of ligated probes
   - Cool down to room temperature, open tubes
   - Add 10 µl Polymerase master mix at room temperature
   - Start PCR

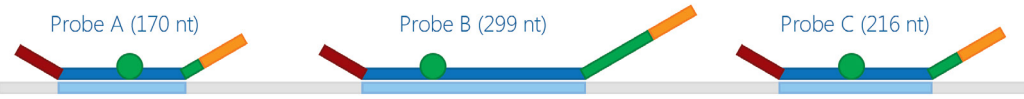
5. Fragment separation by capillary electrophoresis

How MLPA® works

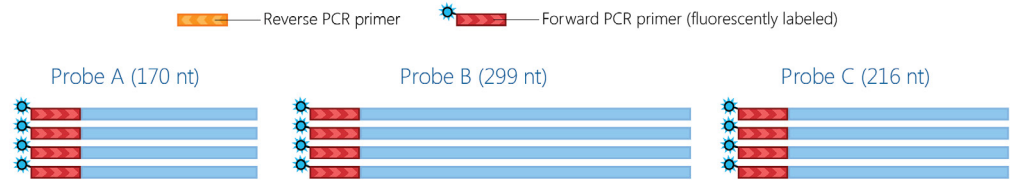
1. Denaturation/2. Hybridisation: Left (LPO) and Right Probe Oligo (RPO) bind to their target DNA.



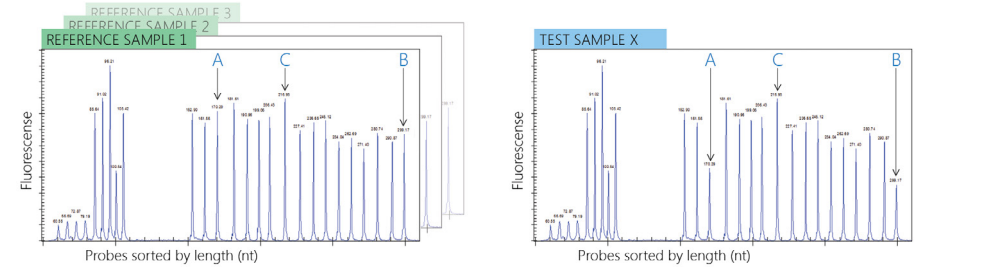
3. Ligation: Hybridised probe oligos are ligated by ligase enzyme.



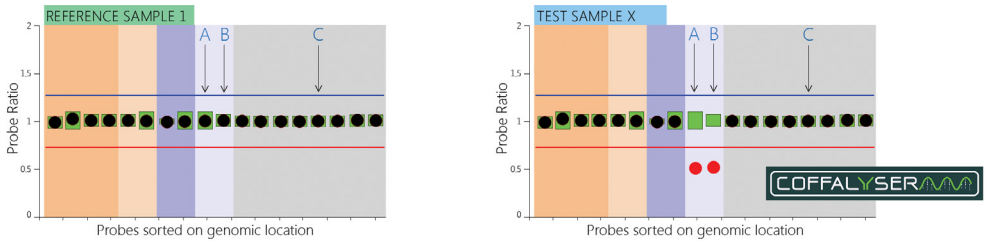
4. Amplification: Ligated probes are amplified using a single primer pair.



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.



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Probemixes: Hematologic Disorders

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 hereditary blood disorders and blood cancers. See www.mlpa.com for a complete overview.

Hereditary Blood Disorders

Application	Probemix	Genes/region
Agammaglobulinemia	P210	BTK
Antithrombin (III) Deficiency	P227	SERPINC1
Diamond-Blackfan Anemia	P212	RPL5, RPL11, RPL35A, RPS17, RPS19, RPS26
Factor Deficiencies	P178	F8
	P207	F7, F8, F9
	P243	F12, SERPING1
	P440	F10, F11
	P469	F5
Familial MDS-AML	P437	GATA2 (+R398W/T354M), TERC, TERT, CEBPA, RUNX1
Fanconi Anemia	P031-P032	FANCA
	P057	FANCD2, PALB2
	P113	FANCB
	P260	PALB2, RAD50, RAD51C, RAD51D
FCGR	P110-P111	FCGR2A (+131R/131H), FCGR2B (+232I/232T), FCGR2C (+ -386G/C), FCGR3A (+158V/158F), FCGR3B, NA1, NA2, SH
Hemolytic Uremic Syndrome, atypical (aHUS)	P236	CFH, CFHR1/2/3/5
	P296	CD46, CFI
Hereditary Non-Spherocytic Haemolytic Anemia	P203	PKLR
Hyper-IgE recurrent Infection Syndrome (HIES)	P385	DOCK8
	P386	DOCK8, STAT3
Macrothrombocytopenia	P432	MYH9
Protein C Deficiency	P265	PROC
Protein S Deficiency	P112	PROS1
Thalassemias	P013	ATRX
	P102	HBB
	P140	HBA1, HBA2
von Willebrand Disease	P011-P012	VWF

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 point 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.