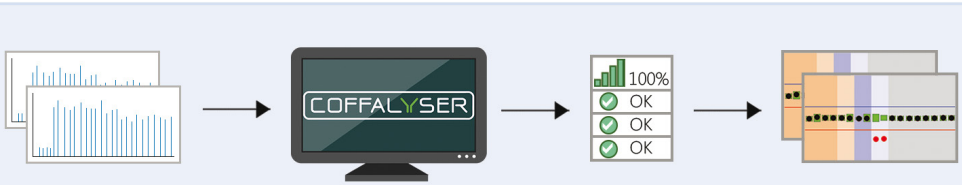
SALSA® MLPA®
Hematologic Disorders

MRC Holland

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

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.

MRC Holland

Willem Schoutenstraat 1
1057 DL Amsterdam
the Netherlands

tel +31 88 865 7200
info@mrcholland.com
www.mrcholland.com

SB-M-HD-010D

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. 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

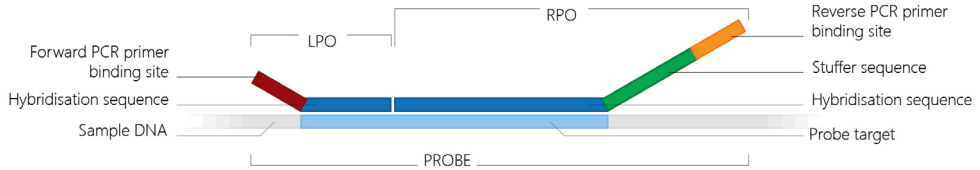
4. PCR amplification of ligated probes
- Ligated MLPA probes are amplified by adding SALSA Polymerase and a single fluorescently-labelled primer pair

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

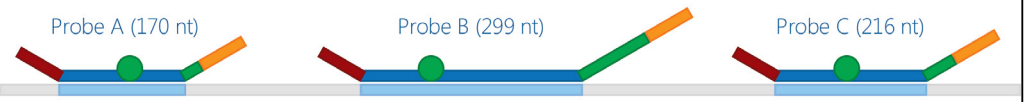
6. Analysis by Coffalyser.Net

How MLPA® works

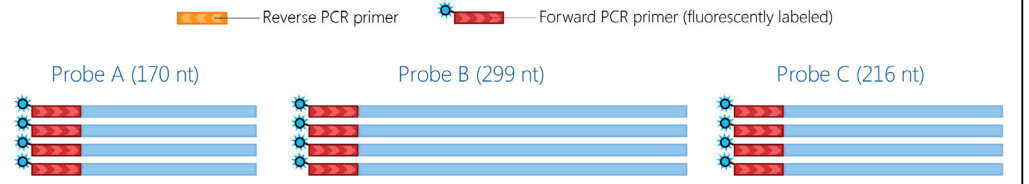
1. Denaturation/2. Hybridisation: Left (LPO) and Right Probe Oligo (RPO) bind to their target DNA.



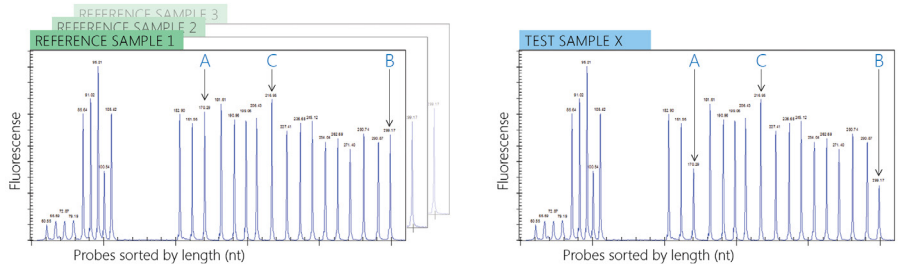
3. Ligation: Hybridised probe oligos are ligated by ligase enzyme.



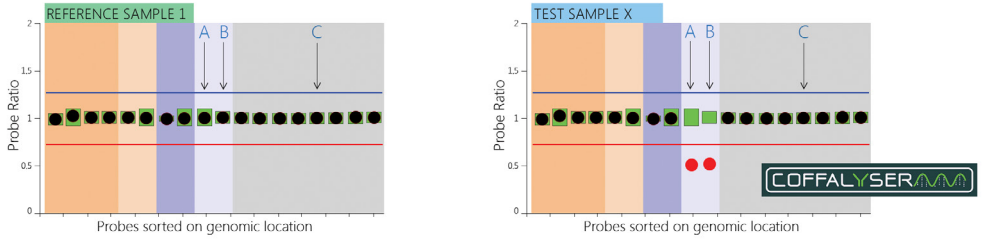
4. Amplification: Ligated probes are amplified using a single primer pair.



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: Hematologic Disorders

Over 350 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.mrcholland.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/2B*), Xp22.33 PAR1 region
	P383	*STIL-TAL1, LEF1, CASP8AP2, MYB, EZH2, CDKN2A/2B, MTAP, MLLT3, NUP214-ABL1, PTEN, LMO1, LMO2, NF1, SUZ12, PTPN2, PHF6*
	ME024	*CDKN2A/2B*, MTAP, MIR31, CDKN2B-AS1*, PAX5*
Chronic Lymphoblastic Leukaemia (CLL)	P037	11q22.3 (*ATM*), chr. 12, 13q14, 17p13 (*TP53*), 2p, 6q, 8, 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/2B*), 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/2B, 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.