



SALSA® MLPA® is the method of choice for the detection of copy number alterations (CNAs) in cancer. MRC Holland offers over 60 MLPA assays specifically designed to detect CNAs and selected point mutations in hundreds of genes and regions commonly affected in various cancers. For neoplasms in which DNA methylation plays a role, such as glioma and Lynch syndrome-related cancers, we offer MLPA assays that combine genomic analysis with easy bisulfite-free methylation profiling.

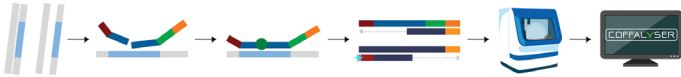
digitalMLPA™, a more recent adaptation of the MLPA technology, combines the robustness and simplicity of MLPA with the high throughput of NGS platforms. With our digitalMLPA NXtec™ assays, a larger number of targets can be analysed in one reaction, providing a comprehensive cancer genetic profile.

Both technologies share features that make them well-suited for cancer applications:

- ▶ Unparalleled copy number detection.
- ▶ Wide detection range – from chromosomes to single exons.
- ▶ High reliability even in complex genomic regions.
- ▶ Free software, simple analysis and clear-cut results.

MLPA

- ✓ Multiplex PCR-based method for targeted CNA, point mutation and methylation detection
- ✓ Needed: thermocycler and capillary electrophoresis device
- ✓ 40-60 targets per reaction
- ✓ 50 ng of DNA input per sample

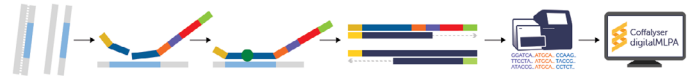


Top-Selling Applications

Colon cancer (Lynch Syndrome)	ME011 Mismatch Repair Genes
Acute Lymphoblastic Leukemia	P335 ALL-IKZF1 P202 IKZF1-ERG P327 iAMP21-ERG P383 T-ALL
Gliomas	P088 Oligodendroglioma ME012 MGMT-IDH-TERT P105 Glioma P370 BRAF-IDH1-IDH2

digitalMLPA

- ✓ NGS-based MLPA for targeted CNA and point mutation detection
- ✓ Needed: thermocycler and Illumina sequencing platform
- ✓ 600-1200 targets per reaction
- ✓ 20 ng of DNA input per sample



Highlighted Applications

Multiple Myeloma	NXtec D006 Multiple Myeloma – 1p, 1q, 13q, 17p (<i>TP53</i>), trisomies (3, 5, 7, 9, 11, 15, 19, 21)
Acute Lymphoblastic Leukemia	NXtec D007 Acute Lymphoblastic Leukemia – 73 ALL-related genes and 8 ALL-related regions; hyperdiploidy and hypodiploidy

SALSA® FFPE Solution

for reliable MLPA results on FFPE material

- ✓ Low-cost reagent for extraction, no costly columns
- ✓ Limited hands-on time
- ✓ Simple extraction steps performed in one tube
- ✓ No deparaffinization step with xylene required
- ✓ High DNA yield

SALSA® FFPE Solution (SFS) was created to reduce the challenges that come when working with FFPE material. The SFS method yields DNA that gives reliable MLPA results using crude tissue lysates. When optimal tissue fixation and DNA extraction methods are chosen, FFPE extracted DNA can be well suited for use with many of our MLPA assays and other molecular applications.

General Tumour Profiling

SALSA® MLPA® Probemix	Target genes/regions
ME001 Tumour suppressor mix*	Tumour suppressor gene methylation profiling
ME024 9p21 CDKN2A/2B region*	CDKN2A/2B, MTAP, MIR31, PAX5 and methylation profiling of CDKN2A/2B
P175 Tumour Gain	Multiple genes/regions
P181 Centromere mix 1 P182 Centromere mix 2	Centromeres
P298 BRAF-HRAS-KRAS-NRAS	BRAF, HRAS, KRAS, NRAS
P323 CDK4-HMGA2-MDM2	12p, 12q, GLI1, CDK4, HMGA2, MDM2
P474 CD274-PDCD1LG2-JAK2	CD274 (PD-L1), PDCD1LG2 (PD-L2), JAK2

Leukemia & Lymphoma

digitalMLPA assay	Target genes/regions
NXtec D006 Multiple Myeloma	1p (FAF1, CDKN2C, DAB1, EVI5, RPL5, CDC14A, TENT5C), 1q (ANP32E, MCL1, ADAR, CKS1B, SLAMF7, PBX1), 4p (FGFR3, NSD2), 11q (CCND1, BIRC2/3, ATM), 12p, 13q, 14q, 16q (CYLD, WWOX), 17p (TP53), 20q (MAFB), Xp (KDM6A), trisomies, BCMA, CRBN, GPRC5D, FcRH5, IKZF1/3, IRF4, MYC, RPL5, SLAMF7, BRAF (p.V600E)
NXtec D007 Acute Lymphoblastic Leukemia	ADD3, BACH2, BCL2L1, BTG1, BTLA, CASP8A2, CD200, CDKN1B, CDKN2A, CDKN2B, CDX2, CREBBP, CTCF, DMD, DNMT3A, DNMT3B, EPHA1, EPHA7, EPOR, ERG, ETV6, EZH2, FHIT, FLT1, FLT3, IGHM, IKZF1, IKZF2, IKZF3, JAK2, KRAS, LEFT1, LMO1, MAP3K7, MEF2C, MLLT3, MTAP, MYB, NF1, NOTCH1, NR3C1, NR3C2, PAN3, PAX5, PHF6, PRAMENP, PTEN, PTPN2, RB1, RUNX1, SPRED1, SUZ12, TBL1XR1, TOX, TP53, VPREB1, 5q, 6q15-q16.1, 13q12.2-q12.3, 17q21, 20q11.21 (dic(9:20) breakpoint), 21q (iAMP21), 22q11.22, PAR1 region and fusion indications (MEF2D-BCL9, MEF2D-HNRNP1, MEF2D-NRNPUL1, MEF2D-DAZAP1, NUP214-ABL1, PDGFRB-EBF1, RAG2-LMO2, STIL-TAL1, TCF3-HLF, TCF3-PBX1, UBTX-ATXN7L3)

SALSA® MLPA® Probemix	Target genes/regions
P037 CLL-1	2p (MYCN, ALK, REL), 6q (TNFAIP3), 8p (TNFRSF10A/B), 8q (EIF3H, MYC), 9p21 (CDKN2A/B), 11q (ATM), chromosome 12, 13q14 (MIR15A, DLEU2/7), 17p (TP53)
P038 CLL-2	10q23 (PTEN), 11q (ATM), chromosome 12, 13q14 (RB1, DLEU1/2), 14q, 17p13 (TP53), chr. 19 and NOTCH1 (p.P2514Rfs*4), SF3B1 (p.K700E), MYD88 (p.L265P)
P040 CLL	11q13-q25, chr. 12, 13q14, 17p13 (TP53)
P041 ATM-1 / P042 ATM-2	ATM
P047 RB1*	RB1+ flanking, RB1 methylation profiling
P056 TP53	TP53, CHEK2 (+c.del1100C)
P202 IKZF1-ERG	IKZF1, ERG, CDKN2A/2B, 14q32
P323 CDK4-HMGA2-MDM2	12p, 12q (GLI1, CDK4, HMGA2, MDM2)
P327 iAMP21-ERG	iAMP21, RUNX1, ERG
P329 CLR2-CSF2RA-IL3RA	Xp22.33 PAR1 region (SHOX, CRLF2, CSF2RA, IL3RA)
P335 ALL-IKZF1	IKZF1, PAX5, ETV6, RB1, BTG1, EBF1, 9p21.3 (CDKN2A/2B), Xp22.33 PAR1 region
P377 Hematologic Malignancies	2p (MYCN, ALK), 5q (MIR145, EBF1, MIR146A), 6q, 7p12 (IKZF1), 7q, 8q24 (MYC), 9p (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), JAK2 (p.V617F)
P383 T-ALL	STIL-TAL1, LEFT1, CASP8A2, MYB, EZH2, CDKN2A/2B, MTAP, MLLT3, NUP214-ABL1, PTEN, LMO1, LMO2, NF1, SUZ12, PTPN2, PHF6
P414 MDS	Chr. 3, 5q, 7q, 8q, 11q (KMT2A), 12p (ETV6), chr. 17 (TP53), chr. 19, 20q, chr. Y and JAK2 (p.V617F)
P419 CDKN2A/2B-CDK4	CDKN2A/2B, CDK4, MTAP, MITF (p.E318K)
P420 MPN mix 1	Point mutation detection with only >10% mutation burden for JAK2 (p.V617F, p.E543_D544del, p.N542_E543del), MPL (p.W515L, p.W515K), KIT (p.D816V), CALR (p.L367fs*46, p.K385fs*47)
P425 Multiple Myeloma	1p12-p32, 1q21-q23, 5q31, chr. 9, 12p13, 13q14, 14q32 (TRAF3), chr. 15, 16q12-q23 (CYLD, WWOX), 17p13 (TP53)
P437 Familial MDS-AML	GATA2 (+p.R398W, p.T354M), TERC, TERT (+p.A1062T), CEBPA, RUNX1
P496 KMT2A	5q (CTNNA1, NPM1), 7p (IKZF1), 7q (CUX1, KMT2E, EZH2), 11q (ATM and KMT2A PTDs), 17p (TP53, NF1), ASXL1 (c.1934dupG)
P520 MPN mix 2	Point mutation detection with only >1% mutation burden for JAK2 (p.V617F, p.E543_D544del, p.N542_E543del), MPL (p.W515L, p.W515K), KIT (p.D816V), CALR (p.L367fs*46, p.K385fs*47)

Solid Tumours

SALSA® MLPA® Probemix	Target genes/regions
ME011 Mismatch Repair Genes*	MLH1 methylation, BRAF p.V600E point mutation and associated Lynch syndrome genome changes profiling
ME012 MGMT-IDH-TERT*	Methylation profiling of MGMT, IDH1 (p.R132H/C), IDH2 (p.R172K/M), TERT (C228T, C250T)
ME042 CIMP*	Methylation profiling of CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3, SOCS1
ME053 BRCA1-BRCA2-RAD51C	Methylation profiling of BRCA1, BRCA2, RAD51C
P027 Uveal melanoma	1p, chr. 3, chr. 6, chr. 8
P044 NF2	NF2
P047 RB1*	RB1+ flanking, RB1 methylation profiling
P056 TP53	TP53, CHEK2 (+c.del1100C)
P078 Breast tumour	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)
P083 CDH1	CDH1
P088 Oligodendroglioma 1p-19q	1p, 9p21, 19q, IDH1 (p.R132H/C), IDH2 (p.R172K/M)
P105 Glioma	TERT (C228T, C250T), chr. 7 (EGFR), chr. 10 (PTEN), CDKN2A, CDK4, MIR26A2, MDM2, NFKBIA, PDGFRA, TP53
P225 PTEN	PTEN, PTENP1
P244 AIP-MEN1-CDKN1B	AIP, MEN1, CDKN1B
P251 NB mix 1 P252 NB mix 2 P253 NB mix 3	Chr. 1, 2 (NBAS, DDX1, MYCN, ALK, BMPR2), 3, 4, 7, 9 (PTPRD, CDKN2A), 11, 12, 14q, 17 (TP53, WSB1)
P258 SMARCB1	SMARCB1
P298 BRAF-HRAS-KRAS-NRAS	BRAF, HRAS, KRAS, NRAS
P301 Medulloblastoma mix 1 P302 Medulloblastoma mix 2 P303 Medulloblastoma mix 3	Chr. 1, 2, 3, 4q, 5q, 6, 7, 8, 9, 10, 14q, 16, 17, 20
P308 MET	MET, PTEN, LRRK2
P315 EGFR	EGFR (+p.L858R, p.T790M)
P323 CDK4-HMGA2-MDM2	12p, 12q (GLI1, CDK4, HMGA2, MDM2)
P370 BRAF-IDH1-IDH2	3p (SRGAP3-RAF1), 6q (MYB), 7q (KIAA1549-BRAF), 8p (FGFR1-TACC1), 8q (MYBL1), 9p21, IDH1 (p.R132H/C), IDH2 (p.R172K/M), BRAF (p.V600E)
P378 MUTYH	MUTYH, SCG5, GREM1
P380 Wilms' tumour	1p/q, 2p (MYCN), 2q, 4q (FBXW7), 11p (WT1), 16p/q, 17p (TP53), Xq11 (AMER1)
P417 BAP1	BAP1
P419 CDKN2A/2B-CDK4	CDKN2A/2B, CDK4, MITF (p.E318K)
P429 SDHA-MAX-TMEM127	SDHA, MAX, TMEM127
P433 ARID1A-ARID1B	ARID1A, ARID1B
P455 LZTR1	LZTR1
P466 CDC73	CDC73
P472 SUFU	SUFU
P477 Head and neck carcinoma	Multiple genes/regions
P478 SMARCE1	SMARCE1
P481 PRKAR1A-ARMC5	PRKAR1A, ARMC5
P482 DICER1	DICER1
P483 HER gene family	EGFR, ERBB2, ERBB3, ERBB4

* This probemix also includes probes that determine the methylation status of a target.



Confidence in Copy Number Determination