SALSA MLPA probemix ME002-C1 Tumour suppressor mix 2

Lot C1-1115, C1-0412. As compared to the previous version (lot B1-0809), probes for KLLN/PTEN and MGMT promoter regions have been replaced, and lengths of several probes have been adjusted. Furthermore, the control fragments have been changed (QDX2).

This SALSA® MLPA® probemix is for basic research! This probemix enables you to detect aberrant methylation of CpG islands upstream of genes for which an altered methylation status in one or more types of tumours has been reported in literature. In case interesting results are obtained by users, it is possible to develop methylation probemixes specific for a certain tumour in collaboration with MRC-Holland. Interpretation of results obtained with this product can be complicated. MRC-Holland cannot provide assistance with data interpretation.

Aberrant methylation of CpG-islands has been shown to be associated with transcriptional inactivation of tumour suppressor genes in a wide spectrum of human cancers. CpG-islands are located in or near the promoter region or other regulatory regions of approximately 50% of human genes.

This ME002-C1 MS-MLPA probemix contains 27 MS-MLPA probes which detect the methylation status of promoter regions of 25 different tumour suppressor genes. These tumour suppressor genes are frequently silenced by methylation in tumours, but are unmethylated in blood-derived DNA of healthy individuals. In addition, 14 reference probes are included which are not affected by HhaI digestion. Besides detecting aberrant methylation, all 41 probes present will give information on copy number changes in the analysed sample. The MLPA reaction requires as little as 20 ng of human DNA and can be used on a variety of DNA samples, including those derived from paraffin-embedded tissues.

The MS-MLPA probes in this ME002-C1 probemix detect sequences in promoter regions of tumour suppressor genes that are unmethylated in most blood-derived DNA samples. Upon digestion, the peak signal obtained in unmethylated samples will be very small or absent. In contrast, when tested on *in vitro* methylated human DNA, these probes do generate a signal. We have no data showing that methylation detected by a particular probe indeed influences the corresponding mRNA levels.

This SALSA® MS-MLPA® probemix can be used to detect *aberrant methylation* of one or more sequences of the tumour suppressor genes. Methylation levels can be different for different tissues. If possible, use identically treated test and reference samples (same tissue type and extraction method). This SALSA® MS-MLPA® probemix can also be used to detect *deletions/duplications* of one or more sequences in the above mentioned chromosomal regions in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings. Finally, note that most defects in this gene are expected to be small (point) mutations which will not be detected by this SALSA® MS-MLPA® test. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons.

SALSA® MS-MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MS-MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of this SALSA® MS-MLPA® probemix and reagents requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002). The MS-MLPA method for the detection of both copy numbers and methylation changes was described in Nucleic Acid Research 33, e128 by Nygren et al. 2005.

More information

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Related SALSA® MLPA® probemixes

- ME001/004 Tumour suppressor mix 1/4. Contain probes for various tumour suppressor genes.
- ME012 MGMT-IDH1-IDH2. Contains six MS-MLPA probes for MGMT gene promoter and probes specific for IDH1 (R132H/C) and IDH2 (R172K/M).

More methylation-specific probemixes are available, please enquire.

References of SALSA® MS-MLPA® probemixes ME001/ME002 Tumour suppressor mix 2

- Branham MT et al. (2016) Epigenetic regulation of ID4 in the determination of the BRCAness phenotype in breast cancer. *Breast Cancer Res Treat*. 155:13-23.
- Sahnane N et al. (2015) Microsatellite unstable gastrointestinal neuroendocrine carcinomas: a new clinicopathologic entity. *Endocr Relat Cancer*. 22:35-45.
- Martignoni G et al. (2014) Renal cell carcinoma with smooth muscle stroma lacks chromosome 3p and VHL alterations. *Mod Pathol*. 27:765-74.
- Rankeillor KL et al. (2014) Methylation-specific multiplex ligation-dependent probe amplification identifies promoter methylation events associated with survival in glioblastoma. *J Neurooncol*. 117:243-51.
- Furlan D et al. (2014) APC alterations are frequently involved in the pathogenesis of acinar cell carcinoma of the pancreas, mainly through gene loss and promoter hypermethylation. *Virchows Arch*. 464:553-64
- Appelqvist F et al. (2014) Deletion of the MGMT gene in familial melanoma. *Genes Chromosomes Cancer*. 53:703-11.
- Feierabend D et al. (2014) Methylation-specific multiplex ligation-dependent probe amplification and its impact on clinical findings in medulloblastoma. *J Neurooncol*. 116:213-20.
- Stefanoli M et al. (2014) Prognostic relevance of aberrant DNA methylation in g1 and g2 pancreatic neuroendocrine tumors. *Neuroendocrinology*. 100:26-34.
- Sacristan R et al. (2014) Molecular classification of non-muscle-invasive bladder cancer (pTa low-grade, pT1 low-grade, and pT1 high-grade subgroups) using methylation of tumor-suppressor genes. *J Mol Diagn*. 16:564-72.
- Furlan D et al. (2013) Diagnostic utility of MS-MLPA in DNA methylation profiling of adenocarcinomas and neuroendocrine carcinomas of the colon-rectum. *Virchows Arch*. 462:47-56.
- La Rosa S et al. (2013) Mixed exocrine-neuroendocrine carcinoma of the nasal cavity: clinico-pathologic and molecular study of a case and review of the literature. *Head Neck Pathol*. 7:76-84.
- Moelans CB et al. (2012). Frequent promoter hypermethylation of BRCA2, CDH13, MSH6, PAX5, PAX6 and WT1 in ductal carcinoma in situ and invasive breast cancer. *J Pathol*. 255:222-3.
- Cabello MJ et al. (2011) Multiplexed Methylation Profiles of Tumor Suppressor Genes in Bladder Cancer. *J Mol Diagn*. 13:29-40.

Note: These are the most relevant references for this probemix; PubMed and Google Scholar provide more references and information on the use of the ME002 probemix.

Methylation-specific MLPA

Please note that each MS-MLPA reaction generates two samples that need analysis by capillary electrophoresis: one undigested sample for copy number detection and one digested sample for methylation detection.

A modification of the MLPA technique, MS-MLPA allows the detection of both copy number changes and methylation levels of 10-50 different sequences in one simple reaction. MLPA probes for methylation quantification are similar to normal MLPA probes, except that the sequence detected by the MS-MLPA probe contains the sequence recognised by the methylation-sensitive restriction enzyme HhaI.
Similar to ordinary MLPA reactions, the MS-MLPA protocol starts with sample DNA denaturation and overnight hybridization. The reaction then is split into two tubes. One tube is processed as a standard MLPA reaction. This reaction provides information on copy number changes. The other tube of the MLPA hybridization reaction is incubated with the methylation-sensitive HhaI endonuclease while simultaneously, the hybridised probes are ligated. Hybrids of (unmethylated) probe oligonucleotides and unmethylated sample DNA are digested by the HhaI enzyme. Digested probes will not be exponentially amplified by PCR and hence will not generate a signal when analysed by capillary electrophoresis. In contrast, if the sample DNA is methylated, the hemi-methylated probe-sample DNA hybrids are prevented from being digested by HhaI and the ligated probes *will* generate a signal.

The MS-MLPA technique should always be internally validated before use in your laboratory. Results of MS-MLPA are highly dependent on the HhaI enzyme used. HhaI enzymes that are resistant to heat inactivation are NOT compatible with the MS-MLPA technique and will give aberrant results. These include, but may not

be limited to, Thermo Fisher Scientific enzymes HhaI, ANZA 59 HhaI, and FastDigest HhaI. We recommend using Promega's HhaI enzyme (R6441) as this is the only restriction enzyme that has been validated for use with MS-MLPA by MRC-Holland.

More information about MS-MLPA can be found in the MS-MLPA protocol.

Please note that this product can not be used with an alternative protocol in which the genomic DNA is first digested with HhaI, followed by MLPA reactions on both digested and undigested genomic DNA.

Data analysis

The ME002-C1 Tumour suppressor mix 2 probemix contains 41 MLPA probes with amplification products between 136 and 484 nt. In addition, it contains 9 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA Denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

The analysis of MS-MLPA probemixes consists of two parts: 1) determining copy numbers by comparing different undigested samples, and 2) determining methylation patterns by comparing each undigested sample to its digested counterpart (MS-MLPA probemixes only). The second part is unique for MS-MLPA probemixes and serves to semi-quantify the percentage of methylation within a given sample.

1) Copy number analysis

- Selection of reference probes
First select suitable reference probes for copy number detection. These are probes detecting relatively quiet regions in the particular type of tumour studied. The reference probes selected will therefore depend on the application.

- Intra-sample data normalisation
For analysis of MLPA results, not the absolute fluorescence values but "intra-normalized" data are used (relative peak heights). The data generated in the undigested sample should first be normalized intra-sample by dividing the signal of each probe by the signal of every reference probe in that sample, thus creating as many ratios per probe as there are reference probes. Subsequently, the median of all these produced ratios per probe should be taken; this is the probe's Normalisation Constant. This Normalisation Constant can then be used for sample to reference sample comparison.

- Inter-sample normalisation (comparison with reference samples)
The final probe ratio, or ploidy status, of each probe in each sample is calculated by dividing a) the Normalisation Constant of each probe obtained on the undigested test sample by b) the average Normalisation Constant of that probe obtained on the undigested reference samples.

Please note that for samples containing both tumour and normal cells, MLPA experiments will indicate the average copy number of genes!

2) Methylation analysis

- Selection of reference probes
Use the reference probes for methylation as marked in Table 1. All reference probes used for methylation analysis do not contain a HhaI site.

- Intra-sample data normalisation
For analysis of MLPA results, not the absolute fluorescence values but "intra-normalized" data are used (relative peak heights). The data generated in the digested sample should first be intra-sample normalized by dividing the signal of each probe by the signal of every reference probe in that sample, thus creating as many ratios per probe as there are reference probes. Subsequently, the median of all these produced ratios per probe should be taken; this is the probe's Normalisation Constant. This Normalisation Constant can then be used for sample to reference sample comparison.

- Methylation analysis (comparison with reference samples)
The methylation status of each MS-MLPA probe* in each sample is calculated by dividing a) the Normalisation Constant of each probe obtained on the digested test sample by b) the Normalisation Constant of each MS-MLPA probe obtained on the corresponding undigested sample. Multiplying this value by 100 gives an estimation of the percentage of methylation. Aberrant methylation can then be identified by comparing the methylation status of one or more MS-MLPA probes in the sample in question to that obtained on reference samples.

Data normalisation should be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blotting, long range PCR, qPCR, FISH.

Some probes, such as those for CDH13 (219 nt), WT1 (247 nt), ESR1 (301 nt), and MSH6 (328 nt), show background signals in DNA derived from blood, indicating methylation in some cells, but NOT in DNA from some other tissues! These background signals may vary between 5-20%!

Warning: MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a sample. Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. When in doubt, users should always verify the latest updates of the database and scientific literature when interpreting their findings.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

This probemix was developed at MRC-Holland.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1. SALSA MS-MLPA ME002-C1 probemix

Length (nt)	SALSA MLPA probe	HhaI site	Expected signal reduction ᵛ	Chromosomal position	Reference probe for	
					Copy number	Methylation
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA					
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation					
100	X-fragment: Specific for the X chromosome					
105	Y-fragment: Specific for the Y chromosome					
136	CREM probe 00981-L00566	-		10p11.21	Yes	Yes
141	BRCA1 probe 03296-L01269	+	100%	17q21.31	Yes	-
148	BRCA2 probe 02285-L01776	+	100%	13q13.1	Yes	-
154 Δ	CFTR probe 02944-L02376	-		7q31.2	Yes	Yes
161 ¥	ATM probe 03023-L23862	+	100%	11q22.3	Yes	-
167 ¥	TP53 probe 18348-L23289	+	100%	17p13.1	Yes	-
176 ¥	PTCH1 probe 03708-L23221	-		9q22.32	Yes	Yes
183 * «	KLLN probe 13686-L15155	+	100%	10q23.31	Yes	-
190 «	MGMT probe 05670-L05146	+	100%	10q26.3	Yes	-
202	MLH3 probe 01245-L00793	-		14q24.3	Yes	Yes
210 ¥	PAX5 probe 03750-L23113	+	100%	9p13.2	Yes	-
219 ‡	CDH13 probe 02257-L01742	+	90%	16q23.3	Yes	-
232 ¥	PAH probe 02334-L21324	-		12q23.2	Yes	Yes
240 ¥ «	TP73 probe 16004-L23287	+	100%	1p36.32	Yes	-
247 ¥	WT1 probe 18347-L23288	+	95%	11p13	Yes	-
256	PMP22 probe 01462-L00927	-		17p12	Yes	Yes
265	VHL probe 03818-L03850	+	100%	3p25.3	Yes	-
274 ¥	GSTP1 probe 18345-L23787	+	100%	11q13.2	Yes	-
281 «	TSC2 probe 01832-L01397	-		16p13.3	Yes	Yes
294 ¥ «	CHFR probe 18344-L23785	+	100%	12q24.33	Yes	-
301 ‡	ESR1 probe 02746-L02173	+	90%	6q25.1	Yes	-
310	CDK6 probe 03184-L02523	-		7q21.2	Yes	Yes
319 ¥	RB1 probe 02734-L23112	+	100%	13q14.2	Yes	-
328 ‡	MSH6 probe 01250-L00798	+	85%	2p16.3	Yes	-
337	APC probe 01700-L01341	-		5q22.2	Yes	Yes
346 *	MGMT probe 18346-L23286	+	100%	10q26.3	Yes	-
355	THBS1 probe 01678-L17140	+	100%	15q14	Yes	-
364	CADM1 probe 03816-L17141	+	100%	11q23.3	Yes	-
373	PTEN probe 03638-L17142	-		10q23.31	Yes	Yes
382 «	STK11 probe 06783-L17143	+	100%	19p13.3	Yes	-
391 ¥	KLK3 probe 00713-L23223	-		19q13.33	Yes	Yes
398	PYCARD probe 02252-L01737	+	100%	16p11.2	Yes	-
409	PAX6 probe 03749-L03209	+	100%	11p13	Yes	-
418	ATM probe 02670-L02137	-		11q22.3	Yes	Yes
425 ¥	CDKN2A probe 18349-L23290	+	100%	9p21.3	Yes	-
433	GATA5 probe 03752-L06199	+	100%	20q13.33	Yes	-
445	IL2 probe 00627-L00183	-		4q27	Yes	Yes
452	RARB probe 04046-L02172	+	100%	3p24.2	Yes	-
461	CD44 probe 04500-L02761	+	100%	11p13	Yes	-
472	RB1 probe 04502-L02199	+	100%	13q14.2	Yes	-
484	CASR probe 02683-L02148	-		3q21.1	Yes	Yes

* New in version C1 (from lot C1-0412 onwards). The new MGMT probe is located at a distance of 250 nt from the previous probe, and is located in Methylation Hotspot 2, as stated in Qian & Brent (1997) Cancer res. 57:3672-7.

¥ Changed in version C1 (from lot C1-0412 onwards). Small change in length, no change in sequence detected.

Δ More variable. This probe has a high standard deviation.

« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

‡ This probe shows background signal in DNA derived from blood, indicating methylation in some cells, but NOT in DNA from some other tissues! The background signal after HhaI digestion may vary between 5-20%.

ᵛ The expected signal reduction percentages in this column are indications. Some MS-MLPA probes may have a small residual signal after HhaI digestion in normal blood-derived samples.

Table 2. ME002 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Ligation site	MV location (HG18)	Complete sequence detected by the probes, with HhaI site in grey
240 «	16004-L23287	TP73	NM_005427.3; 174 nt after exon 1; 29551 nt before ATG	01-003.559219	GGCTCCCTCGGAGTTGGATCGGCCCCTGGG-ACTTG**GCGC**TCGCGAGAGGCTGGAGCGGCCAGAG
328 ‡	01250-L00798	MSH6	NM_000179.2; 164-165; 9 nt after ATG	02-047.863865	CGGCTGTCGGTATGTCGCGACAG-AGCACCCTGTACAGCTTCTTCCCCAAGTCTCCG**GCGC**
265	03818-L03850	VHL	NM_000551.3; 250-251; 34 nt after ATG	03-010.158544	CGGAGAACTGGGACGAGGCCGAGG-TAG**GCGC**GGAGGAGGCAGGCGTCGAAGAGTACGG
452	04046-L02172	RARB	NM_000965.4; 355 nt before exon 1; 824 nt before ATG	03-025.444383	GGCGGGAGGCGAGCGG**GCGC**A-GGCGGAACACCGTTTTCCAAGCTAAGCCGCCGCAAATA
484	02683-L02148	CASR	NM_000388.3; 2137-2138	03-123.485226	CCAGTGCCTGTAACAAGTGCCCAGATGACT-TCTGGTCCAATGAGAACCACACCTCCTGCATTGCCAAG
445	00627-L00183	IL2	NM_000586.3; 64-65	04-123.596999	CCTCAACTCCTGCCACAATGTACAGG-ATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACT
337	01700-L01341	APC	NM_000038.5; 1753-1754	05-112.192470	CGAGGAATTTGTCTTGGCGAGCAGAT-GTAAATAGTAAAAAGACGTTGCGAGAAGTTGGAAGTG
301 ‡	02746-L02173	ESR1	NM_000125.3; 122-123; 112 nt before ATG	06-152.170607	GCTCGCGTGTCGGCGGGACAT-**GCGC**TGCGTCGCCTCTAACCTCGGGCTGTGCTCTTTT
310	03184-L02523	CDK6	NM_001145306.1; 1244-1245	07-092.085391	GCGTGATTGGACTCCCAGGAGAAGAAGACT-GGCCTAGAGATGTTGCCCTTCCCAGGCAGGCTTTTCA
154	02944-L02376	CFTR	NM_000492.3; 102 nt before exon 1; 234 nt before ATG	07-116.907124	GGGTGGAGAAAGCCGCTAGAGCAAAT-TTGGGGCCGGACCAGGCAGCACTCGGCTTTTAACCTG
425 †	18349-L23290	CDKN2A	NM_058195.3; 191-192; 31 nt after ATG	09-021.984268	ACATGGT**GCGC**AGGTTCTTGGTGACCCTCCGGA-TTCG**GCGCGC**GTGCGGCCCGCCGCGAGTGAG
210 †	03750-L23113	PAX5	NM_016734.1; 214 nt before exon 1; 661 nt before ATG	09-037.024651	CT**GCGC**TCGTCTAAGCAGCGGGGTT-TGCACATGGAGATGTCACAGGCCCC**GCGC**ACA**GCGC**
176	03708-L23221	PTCH1	NM_000264.3; 3165-3166	09-097.260267	GACACCTCAGACTTTGTGGAGGCAATTGAAA-AAGTAAGGACCATCTGCAGCAACTATACGAGCCTGGG
136	00981-L00566	CREM	NM_181571.2; 689-690	10-035.517225	GCTCCTCCACCAGGTGCTACAAT-TGTACAGTACGCAGCACAATCAGCTGATGGCACACAGC
183 § «	13686-L15155	KLLN	NM_001126049.1; 79-78 reverse; 872 nt before ATG	10-089.613066	CCAGGCAGCTACACTGGGCATGCTCAGTAGA-GCCTGCGGCTTGGGGACTCT**GCGC**TCGCACCCAGAGC
373	03638-L17142	PTEN	NM_000314.6; 1436-1437	10-089.682866	CTGTAAAGCTGGAAAGGGACGAACTGGTGTAATGATA-TGTGCATATTTATTACATCGGGGCAAATTTTTAAAGGC
190 + «	05670-L05146	MGMT	NM_002412.3; 314 nt before exon 1; 346 nt before ATG	10-131.155095	GGCAAACTAAGGCACAGAGCCTCA-GGCGGAAGCTGGGAAG**GCGC**CGCCCGGCTTGTAC
346 +	18346-L23286	MGMT	NM_002412.4; 74 nt after exon 1; 152 nt after ATG	10-131.155600	CTCCCTCGGGACGGTGGCAGCCTCGAGTGGT-CCTGCAG**GCGC**CCTCACTTCGCCGTCGGGTGT
409 †	03749-L03209	PAX6	NM_001604.5; 28 nt before exon 1; 4970 nt before ATG	11-031.789463	GGAGCATCCAATCGGCTG**GCGC**G-AGGCCCCG**GCGC**TGCTTTGCATAAAGCAATATTTTGT
247	18347-L23288	WT1	NM_024426.4; 221 nt before exon 1; 412 nt before CTG	11-032.413841	GGGAGGGTTGTGCCACACCGGCCAGCT-GAGA**GCGC**GTGTTGGGTTGAAGAGGAGGGTGTCTCC
461	04500-L02761	CD44	NM_001001391.1; 23-24; 411 nt before ATG	11-035.116991	GGAGAAGAAAGCCAGTGCGTCTCT-GG**GCGC**AGGGGCCAGTGGGGCTCGGAGGCACAGG
274 †	18345-L23787	GSTP1	NM_000852.3; 46 nt before exon 2; 245 nt after ATG	11-067.108109	GGGCAGACT**GCGC**TCACC**GCGC**CTT-GGCATCCTCCCCCGGGCTCCAGCAAACTTTTCTTTGTT
161	03023-L23862	ATM	NM_000051.3; 136-137; 4658 nt before ATG	11-107.598881	GCGGAGACCGCGTGATACTGGAT-**GCGC**ATGGGCATACCGTGCTCTGCGGCTGCTTGGC
418	02670-L02137	ATM	NM_000051.3; 4320-4319 reverse	11-107.660311	GCATATCATAGACCTTGGTAGCAGTCTCTC-TTTGCTGTGCCATCCCACTGTCTCTGGTACCCTCATAG
364	03816-L17141	CADM1	NM_014333.3; 57-58; 73 nt before ATG	11-114.880363	CTGCCCGGACTCCGCCTCCA**GCGC**ATGTCA-TTAGCATCTCATTAGCTGTCCGCTCGGGCTC

Length (nt)	SALSA MLPA probe	Gene	Ligation site	MV location (HG18)	Complete sequence detected by the probes, with HhaI site in grey
232	02334-L21324	PAH	NM_000277.1; 842-843	12-101.795401	GTTTCAGTGCCCTGGTTCCCAAGAA-CCATTCAAGAGCTGGACAGATTTGCCAATCAGATTCTC
294 † «	18344-L23785	CHFR	NM_001161344.1; 118 nt before exon 1; 407 nt before ATG	12-131.974372	CGAGAGTAG**GCGC**GTGGAGG-**GCGC**TCGGCCATCTTTGATCCTGACCAGGCGACTTCG
148	02285-L01776	BRCA2	NM_000059.3; 23 nt after exon 1; 771 nt before ATG	13-031.787801	CGGGTTAGTGGTGGTGGTAGTGGGTT-GGGACGA**GCGC**GTCTTCCGCAGTCCCAGTCCAGCGTG
319	02734-L23112	RB1	NM_000321.2; 354 nt before exon 1; 520 nt before ATG	13-047.775508	CCAAGGAGGGAGAGTG**GCGC**TC-CCGCCGAGGGTGCACTAGCCAGATATTCCCTGCG
472	04502-L02199	RB1	NM_000321.2; 157 nt before exon 1; 323 nt before ATG	13-047.775703	GGATGCCTCCTGGAAG**GCGC**CTGG-ACCCACGCCAGGTTTCCCAGTTTAATTCCTCATGACTT
202	01245-L00793	MLH3	NM_001040108.1; 3553-3554	14-074.578836	GCGACCTTGTTCTTCCTTTCCTTCCGA-GAGCTCGAGCAGAGAGGACTGTGATGAGACAGGATAA
355	01678-L17140	THBS1	NM_003246.2; 54 nt before exon 1; 834 nt before ATG	15-037.660496	TGCCCGGCCGCCGCCCATTGGCCGGAGG-AATCCCCAGGAATGCGA**GCGC**CCCTTTAAAA
281 «	01832-L01397	TSC2	NM_000548.3; 2080-2081	16-002.061786	GAGCCAGAGAGAGGCTCTGAGAAGAAG-ACCAGCGGCCCCCTTTCTCCTCCCACAGGGCCTCCTG
398	02252-L01737	PYCARD	NM_013258.4; 276-277; 190 nt after ATG	16-031.121292	CAAGCTGGTCAGCTTCTACCTGGAGACCT-ACG**GCGC**CGAGCTCACCGCTAACGTGCTGCG
219 ‡	02257-L01742	CDH13	NM_001257.4; 278-279; 20 nt before ATG	16-081.218154	CGTGCATGAATGAAAACGCCGCC-GG**GCGC**TTCTAGTCGGACAAAATGCAGCCGAG
167	18348-L23289	TP53	NM_000546.5; 52-51 reverse; 10905 nt before ATG	17-007.531511	CTGGACGGTGGCTCTAGACTTTTGAGAAGCTCAAA-ACTTTTA**GCGC**CAGTCTTGAGCACATGGGAGGGGAAA
256	01462-L00927	PMP22	NM_000304.2; 230-231	17-015.104707	CCAGAATGCTCCTCCTGTTGCTGAGTA-TCATCGTCCTCCACGTCGCGGTGCTGGTGCTGCTGTTC
141	03296-L01269	BRCA1	NM_007294.3; 67-68; 1321 nt before ATG	17-038.530922	CCCCTTGGTTTCCGTGGCAACGGA-AAA**GCGC**GGGAATTACAGATAAATTAAAACTGCGACT
382 «	06783-L17143	STK11	NM_000455.4; 691-692; 425 nt before ATG	19-001.157467	GGCCTGTGGGATGGGCGGCCCGGAGA-AGACT**GCGC**TCGGCCGTGTTCATACTTGTCCGTGGGC
391	00713-L23223	KLK3	NM_001648.2; 53-54	19-056.050014	CTGTGTCACCATGTGGGTCCCG-GTTGTCTTCCTCACCCTGTCCGTGACGTGGA
433	03752-L06199	GATA5	NM_080473.4; 208 nt before exon 1 reverse; 658 nt before ATG	20-060.484610	CTTGGCGACAAGGACGCACG-ACACGGGGCGGCCA**GCGC**GGAGCCCGGACCAGTG

The HhaI sites are marked in **grey**. Ligation sites are marked with –

† Note that this probe contains multiple HhaI sites. All sites should be methylated in order to see a signal in the digested sample. Therefore, conclusions cannot be drawn on the methylation status of single HhaI sites.
§ The KLLN and PTEN gene share the same promoter, but are transcribed in opposite directions. This probe is located 1110 nt before the PTEN ATG start codon and 78 nt before PTEN exon 1.
+ For MGMT methylation testing we recommend to use the ME012 MGMT-IDH1-IDH2 probemix. Two methylation hotspots near the transcription start site denote silencing of the MGMT gene (Qian, X.C. & Brent, T.P. (1997) Cancer research 57:3672-3677). Between these methylation hotspots is a region which is much less frequently methylated (Fig. 3 of Qian et al.). Included in this ME002-C1 probemix are two methylation-specific MLPA probes for the MGMT region surrounding the transcription and translation start sites. Based on the article of Qian et al., the 190 nt probe appears to be located within a methylation hotspot and shows frequent methylation. The 346 nt probe is located in methylation hotspot 2. This probe is new in version C1.
« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
‡ This probe shows background signal in DNA derived from blood, indicating methylation in some cells, but NOT in DNA from some other tissues! The background signal after HhaI digestion may vary between 5-20%.

Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene.
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide.

Notes: Complete probe sequences are available on request: info@mlpa.com. Please, notify us of any mistakes: info@mlpa.com.

SALSA MS-MLPA probemix ME002-C1 Tumour suppressor mix 2 sample pictures

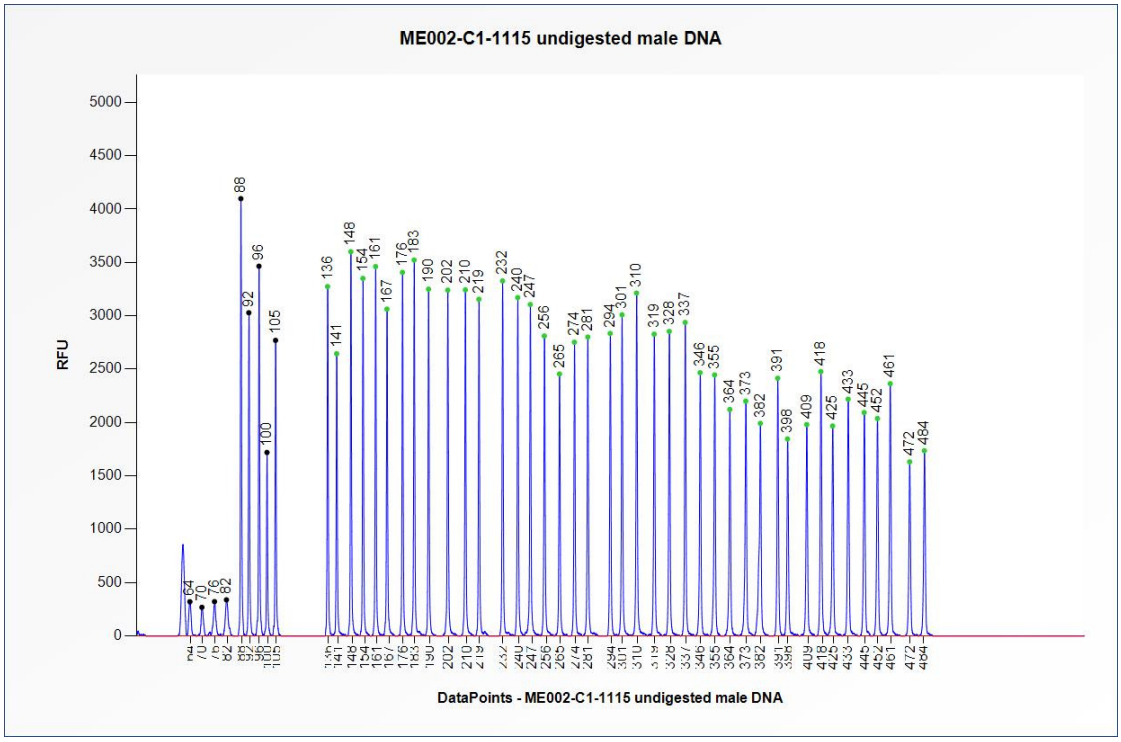

Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng undigested human male control DNA analysed with SALSA® MLPA® probemix ME002-C1 (lot C1-1115) for the quantification of copy numbers.

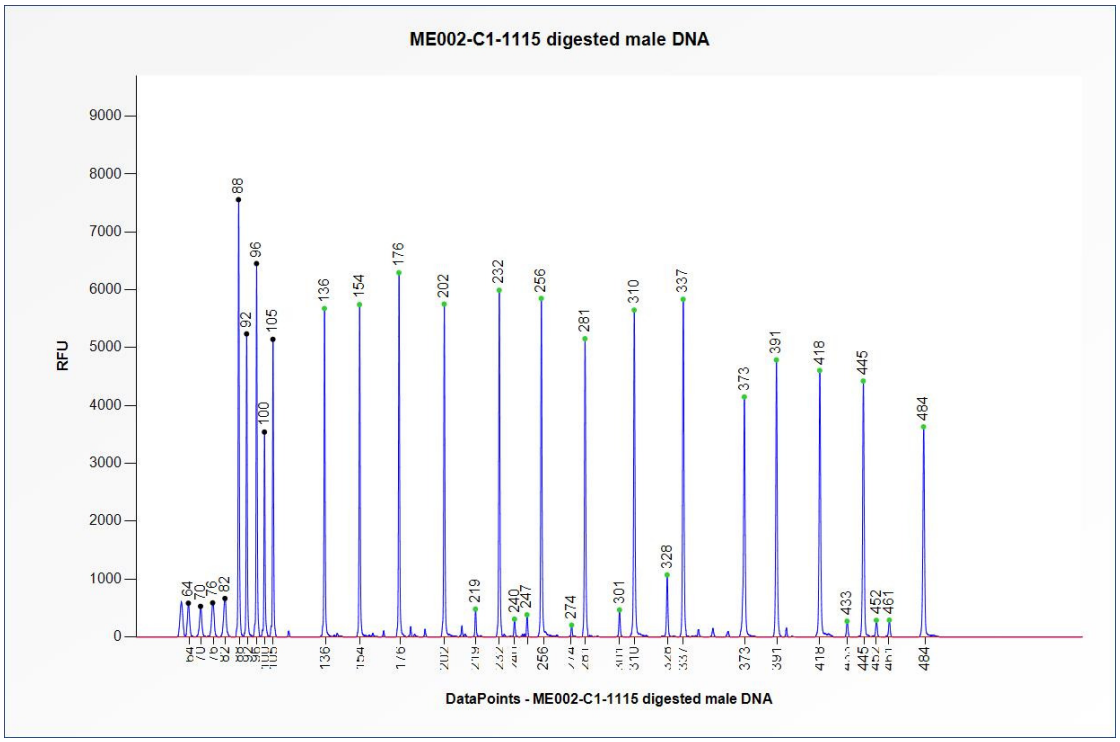

Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng digested human male control DNA analysed with SALSA® MLPA® probemix ME002-C1 (lot C1-1115) to determine the methylation status.

Implemented Changes – compared to the previous product description version(s).

Version 23 – 06 December 2016 (16)
- Warning regarding HhaI enzymes that are resistant to heat inactivation added under Methylation-specific MLPA section.

Version 22 – 20 May 2016 (15)
- Product description adapted to a new product lot (lot number added, changes in Table 1 and Table 2, new pictures included).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- References added on page 2.
- "Expected signal reduction" column and footnote added in Table 1.
- Ligation site information updated in Table 2 for several probes.
- Various minor textual changes.

Version 21 – 19 February 2015 (14)
- Information on chromosomal location for probes at 183 nt, 373 nt and 434 nt corrected in Table 1.
- Related SALSA MLPA probemixes on page 2 modified.
- A new reference added on page 2.

Version 20 (13)
- Location of MGMT gene in table 1 adapted.

Version 19 (09)
- Textual change below Table 2.

Version 18 (09)
- Warning added in Table 1, 191 nt probe 05670-L05146, 241 nt probe 16004-L23287, 281 nt probe 01832-L01397, 293 nt probe 18344-L23785, 382 nt probe 06783-L17143.

Version 17 (08)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- Minor textual changes.

Version 16 (08)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 15 (08)
- Minor textual changes throughout the document.
- Gene name and NM_sequence changed for probe 03808-L02169 in Table 1 and Table 2 and accordingly a note added on page 8.
- New references added on page 2.

Version 14 (08)
- Various textual changes.
- Small correction of chromosomal locations in Table 1.
- Ligation sites of the probes updated according to new version of the NM_reference sequence.

Version 13 (05)
- Warning added below table 2 on the MGMT methylation sensitive probes.

Version 12 (05)
- Data analysis method has been modified.
- Table 1 has been adjusted. Reference probes for methylation and copy number detection are marked in Table 1.

Version 11 (05)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- Minor textual change in page 1, tables have been numbered.

Version 10 (05)
- 'Basic research' note added on page 1
- Columns in Table 2 have been adjusted. Textual changes on page 1 and 2, tables have been numbered.