# SALSA MS-MLPA probemix ME001-C2 Tumour suppressor mix 1

Lot C2-0815 and C2-0412. Compared to previous lot C1-0808, new control fragments have been added (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 ME001-C2 MS-MLPA probemix contains 26 MS-MLPA probes which detect the methylation status of promoter regions of 24 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, 15 reference probes are included which are not affected by HhaI digestion. Besides detecting aberrant methylation, all 41 probes 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 ME001-C2 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 level.

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 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.

**This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license 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**

- ME002 Tumour suppressor mix 2: Can be used for several genes to confirm the results of ME001.
- ME011 Mismatch Repair genes: Contains MS-MLPA probes for promoter regions of MLH1, MSH2, MSH6, PMS2, MSH3 and MLH3 genes.
- ME012 MGMT-IDH1-IDH2: Contains six MS-MLPA probes for MGMT gene promoter and four mutation-specific probes for IDH1 R132H&C and IDH2 R172K&M point mutations.
- ME024 9p21 CDKN2A/2B region: Contains copy number and MS-MLPA probes for CDKN2A/2B gene region, as well as copy number probes for MIR31, MTAP and PAX5 genes.
- More methylation-specific probemixes are available, please enquire.

**References of SALSA® MS-MLPA® probemix ME001 Tumour suppressor mix 1**


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Note: Above is a selection of references for this probemix; PubMed and Google Scholar provide more references and information on the use of the ME001 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 unusual 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 ME001-C2 Tumour suppressor mix 1 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. Probes that are suitable to use for reference in many types of tumour are indicated in Table 1.

*- Intra-sample data normalisation*
For analysis of MLPA results, not the absolute fluorescence values but "intra-normalised" data are used (relative peak heights). The data generated in the undigested sample should first be normalised 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.

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-normalised" data are used (relative peak heights). The data generated in the digested sample should first be normalised 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.

*- 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.

***Note:** An MS-MLPA probe targets a single specific HhaI site in a CpG island; if methylation is absent for a particular CpG-site, this does not necessarily mean that the whole CpG island is unmethylated!

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.

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 ME001-C2 Tumour suppressor mix 1 probemix

| Length (nt) | SALSA MLPA probe | HhaI site | % expected signal reduction | 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 |
| 142 | **TIMP3 probe** 02255-L03752 | + | 100% | 22q12.3 | Yes | - |
| 148 | **APC probe** 01905-L01968 | + | 100% | 5q22.2 | Yes | - |
| 154 | PARK2 probe 03366-L02750 | - | | 6q26 | Yes | Yes |
| 161 | **CDKN2A probe** 01524-L01744 | + | 100% | 9p21.3 | - | - |
| 167 ‡ | **MLH1 probe** 01686-L01266 | + | 100% | 3p22.2 | Yes | - |
| 175 | TNFRSF1A probe 00554-L13516 | - | | 12p13.31 | Yes | Yes |
| 184 | **ATM probe** 04044-L03849 | + | 100% | 11q22.3 | Yes | - |
| 193 | **RARB probe** 04040-L01698 | + | 100% | 3p24.2 | Yes | - |
| 202 | MLH3 probe 01245-L00793 | - | | 14q24.3 | Yes | Yes |
| 211 | **CDKN2B probe** 00607-L00591 | + | 85% | 9p21.3 | - | - |
| 220 « | **HIC1 probe** 03804-L00949 | + | 100% | 17p13.3 | Yes | - |
| 229 | PAH probe 02334-L01820 | - | | 12q23.2 | Yes | Yes |
| 238 « | **CHFR probe** 03813-L03753 | + | 100% | 12q24.33 | Yes | - |
| 246 | **BRCA1 probe** 05162-L04543 | + | 100% | 17q21.31 | Yes | - |
| 256 | BCL2 probe 00587-L00382 | - | | 18q21.33 | Yes | Yes |
| 265 | **CASP8 probe** 02761-L02210 | + | 100% | 2q33.1 | Yes | - |
| 274 « | **CDKN1B probe** 07949-L07730 | + | 100% | 12p13.1 | Yes | - |
| 281 « | TSC2 probe 01832-L01397 | - | | 16p13.3 | Yes | Yes |
| 292 | **KLLN probe** 02203-L08261 | + | 100% | 10q23.31 | Yes | - |
| 301 | **BRCA2 probe** 04042-L03755 | + | 100% | 13q13.1 | Yes | - |
| 310 | CDK6 probe 03184-L02523 | - | | 7q21.2 | Yes | Yes |
| 319 | **CD44 probe** 03817-L01731 | + | 100% | 11p13 | Yes | - |
| 328 | **RASSF1 probe** 02248-L01734 | + | 100% | 3p21.31 | Yes | - |
| 337 | CDH1 probe 02416-L01862 | - | | 16q22.1 | Yes | Yes |
| 346 | **DAPK1 probe** 01677-L01257 | + | 100% | 9q21.33 | Yes | - |
| 353 ∫ | **VHL probe** 03810-L01211 | + | 100% | 3p25.3 | Yes | - |
| 364 ° | **CELF2-region** probe 01234-L00781 | - | | 10p14 | Yes | Yes |
| 373 | **ESR1 probe** 02202-L01700 | + | 100% | 6q25.1 | Yes | - |
| 382 | **RASSF1 probe** 03807-L02159 | + | 100% | 3p21.31 | Yes | - |
| 390 | KLK3 probe 00713-L00108 | - | | 19q13.33 | Yes | Yes |
| 400 « | **TP73 probe** 04050-L01263 | + | 100% | 1p36.32 | Yes | - |
| 409 | **FHIT probe** 02201-L01699 | + | 100% | 3p14.2 | Yes | - |
| 418 | BRCA2 probe 01617-L01199 | - | | 13q13.1 | Yes | Yes |
| 427 ∨ | **CADM1 probe** 03819-L03848 | + | 100% | 11q23.3 | Yes | - |
| 436 | **CDH13 probe** 07946-L07727 | + | 95% | 16q23.3 | Yes | - |
| 444 | CD27 probe 00678-L00124 | - | | 12p13.31 | Yes | Yes |
| 454 | **GSTP1 probe** 01638-L01176 | + | 100% | 11q13.2 | - | - |
| 463 « | **MLH1 probe** 02260-L01747 | + | 100% | 3p22.2 | Yes | - |
| 475 | CTNNB1 probe 03984-L03251 | - | | 3p22.1 | Yes | Yes |
| 484 | CASR probe 02683-L02148 | - | | 3q21.1 | Yes | Yes |

« 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.

‡ Target sequence of this probe contains SNP rs104894994 (C/T) in the GCGC site, 6 nt right from the ligation site. This validated SNP with an allele frequency of 0.068%, when T-allele present, will inhibit the HhaI restriction, resulting in a false-positive methylation signal.

∫ Target sequence of this probe contains SNP rs3087462 (C/T) in the GCGC site, 3 nt right from the ligation site. This validated SNP with an allele frequency of 2.8%, when T-allele present, will inhibit the HhaI restriction, resulting in a false-positive methylation signal.

° Probe renamed (was LOC254312).
∨ Probe renamed (was IGSF4).

**Note:** a non-specific peak is present at ~121 nt. This peak does not correspond to an MLPA probe.

## Table 2. ME001 probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene | Ligation site | MV location (hg18) | (partial) sequence with HhaI site |
|---|---|---|---|---|---|
| 400 ¤ « | 04050-L01263 | **TP73** | NM_005427.4; 12-13; 29790 nt before ATG | 01-003.558980 | CGCCCGCGAAGGGGACGCAGC-GAAACCGGGGCCCGCGCCAGGCCAGCCGGGA |
| 265 | 02761-L02210 | **CASP8** | NM_001080124.1; 100 nt before exon 3 | 02-201.830872 | CTTTCCAATAAAGCATGTCCAGCGCTC-GGGCTTTAGTTTGCACGTCCATGAATTGTCTGCCACA |
| 353 ∫ ¤ | 03810-L01211 | **VHL** | NM_000551.3; 134-135; 80 nt before ATG | 03-010.158427 | GCGAAGACTACGGAGGTCGACTCGGG-AGCGCGCACGCAGCTCCGCCCCGCGTCCGACC |
| 193 ¤ | 04040-L01698 | **RARB** | NM_000965.4; 174 nt before exon 1 | 03-025.444565 | CCGCCGGCTTGTGCGCTCGCT-GCCTGCCTCTCTGGCTGTCTGCTTTTGCAGGGCTGCT |
| 463 + « | 02260-L01747 | **MLH1** | NM_000249.3; 184 nt before exon 1, reverse; 382 nt before ATG | 03-037.009623 | CTGCTGAGGTGATCTGGCGCAGA-GCGGAGGAGGTGCTTGGCGCTTCTCAGGCTCCTCCTCT |
| 167 + ‡ | 01686-L01266 | **MLH1** | NM_000249.3; 186-187; 12 nt before ATG | 03-037.010001 | CGTTGAGCATCTAGACGTTTCCTTGGCTCT-TCTGGCGCCAAAATGTCGTTCGTGGCAGGGGTTATTC |
| 475 | 03984-L03251 | **CTNNB1** | NM_001904.3; 351-352 | 03-041.241067 | GGCTGTTAGTCACTGGCAGCAACA-GTCTTACCTGGACTCTGGAATCCATTCTGGTGCCACT |
| 382 | 03807-L02159 | **RASSF1** | NM_170714.1; 53-52, reverse; 79 nt before ATG | 03-050.353299 | GTCCACAGGGCGGGCCCCGAC-TTCAGCGCCTCCCCCAGGATCCAGA |
| 328 | 02248-L01734 | **RASSF1** | NM_170714.1; 9 nt before exon 1; 141 nt before ATG | 03-050.353349 | CAGTCCCTGCACCCAGGTTTCCA-TTGCGCGGCTCTCCTCAGCTCCTTCCCGCCGC |
| 409 | 02201-L01699 | **FHIT** | NM_002012.2; 65 nt after exon 1 | 03-061.211919 | CGCGGGTCTGGGTTTCCACGC-GCGTCAGGTCATCACCCCGGAGCCCAGTGGG |
| 484 | 02683-L02148 | **CASR** | NM_000388.3; 2137-2138 | 03-123.485227 | CCAGTGCCTGTAACAAGTGCCCAGATGACT-TCTGGTCCAATGAGAACCACACCTCCTGCATTGCCAAGGA |
| 148 | 01905-L01968 | **APC** | NM_000038.5; 71 nt before exon 2 | 05-112.101356 | CAGCTGTGTAATCCGCTGGATGCGGACC-AGGGCGCTCCCCATTCCCGTCGGGAGCCCGC |
| 373 ¤ | 02202-L01700 | **ESR1** | NM_001122742.1; 536-537; 163 nt after ATG | 06-152.170884 | CGCCCGCCGTGTACAACTACCCCG-AGGGCGCCGCCTACGAGTTCAACGCCGCGGC |
| 154 | 03366-L02750 | **PARK2** | NM_004562.2; 990-991 | 06-162.126768 | CGTTCACGACCCTCAACTTGGCTACT-CCCTGCCTTGTGTGGGTAAGTCTAGCATGTTTTCTCTCCAT |
| 310 | 03184-L02523 | **CDK6** | NM_001145306.1; 1244-1245 | 07-092.085392 | GCGTGATTGGACTCCCAGGAGAAGAAGACT-GGCCTAGAGATGTTGCCCTTCCCAGGCAGGCTTTTCA |
| 161 ¤ » | 01524-L01744 | **CDKN2A** | NM_058195.3; 829 nt before exon 1; 989 nt before ATG | 09-021.985277 | CAGAGGGGAAGAGGAAAGAGGAAGAAGCGCTCAGAT-GCTCCGCGGCTGTCGTGAAGGTTAAAACCGAAAATAAAAAT |
| 211 ∧ | 00607-L00591 | **CDKN2B** | NM_078487.2; 470-471; 110 nt after ATG | 09-021.998809 | CTGCGACAGCTCCTGGAAGCCGG-CGCGGATCCCAACGGAGTCAACCGTTTCGGGAGG |
| 346 | 01677-L01257 | **DAPK1** | NM_004938.2; 256 nt after exon 1; 714 nt before ATG | 09-089.303076 | CGCGAGGATCTGGAGCGAACTGCT-GCGCCTCGGTGGGCCGCTCCCTTCCCTCCCT |
| 364 ° | 01234-L00781 | **CELF2-region** | NR_015413.1; 968-967 reverse | 10-011.017024 | CAATTGCCATTTTTTCCTGACATTCACTGT-GGAAATTTGGTGCACGACACTGTTAGGGGAGATCTGT |
| 136 | 00981-L00566 | **CREM** | NM_181571.1; 68-690 | 10-035.517226 | GCTCCTCCACCAGGTGCTACAAT-TGTACAGTACGCAGCACAATCAGCTGATGGCACACAGCAGT |
| 292 ¤ | 02203-L08261 | **KLLN** | NM_001126049.1; 806-805 reverse | 10-089.612349 | CACCGGAGCGGGCGCAGGAGA-GGCCTGCGGGGTGCGTCCCACTCACAGGGAT |
| 319 ¤ | 03817-L01731 | **CD44** | NM_001001391.1; 418-419; 17 nt before ATG | 11-035.117390 | CTCCTTTCGCCCGCGCCCTCC-GTTCGCTCCGGACACCATGGACAAGTTTTGGTGG |
| 454 ¤ | 01638-L01176 | **GSTP1** | NM_000852.3; 153-154 | 11-067.107775 | CGAAGAGCGGCCGGCGCCGTG-ACTCAGCACTGGGGCGGAGCGGGGCGGGACC |
| 184 ¤ | 04044-L03849 | **ATM** | NM_000051.3; 300-301 | 11-107.599045 | GGAGGGAGGAGGCGAGAGGAGTCGGGA-TCTGCGCTGCAGCCACCGCCGCGGTTGATACTACTTT |
| 427 ¤ | 03819-L03848 | **CADM1** | NM_014333.3; 175 nt before exon 1; 305 nt before ATG | 11-114.880586 | CCTGGAGCCCGAGTCCTTGCACGCCA-GGCGCCCGGGAGAACACTTTTTCCTTGATCCGGGGAAAGC |

| Length (nt) | SALSA MLPA probe | Gene | Ligation site | MV location (hg18) | (partial) sequence with HhaI site |
|---|---|---|---|---|---|
| 175 | 00554-L13516 | **TNFRSF1A** | NM_001065.3; 272-273 | 12-006.321242 | GCCACACTGCCCTGAGCCCAA-ATGGGGGAGTGAGAGGCCATAGCTGTCTGGC |
| 444 | 00678-L00124 | **CD27** | NM_001242.4; 898-899; exon 6 | 12-006.430703 | GAAAGTCCTGTGGAGCCTGCA-GAGCCTTGTCGTTACAGCTGCCCCAGGGAGG |
| 274 « | 07949-L07730 | **CDKN1B** | NM_004064.4; 414-415; 157 nt before ATG | 12-012.761864 | AGCCCCTGCGCGCTCCTAGA-GCTCGGGCCGTGGCTCGTCGGGGTCTGTGTCTTT |
| 229 | 02334-L01820 | **PAH** | NM_000277.1; 842-843 | 12-101.795401 | CAGTGCCCTGGTTCCCAAGAA-CCATTCAAGAGCTGGACAGATTTGCCAATCAGATTCTCAG |
| 238 ¤ « | 03813-L03753 | **CHFR** | NM_001161344.1; 116 nt before exon 1; 407 nt before ATG, reverse | 12-131.974373 | CGCGAGAGTAGGCGCGTGGAGG-AGCGCTCGGCCATCTTTGATCCTGACCAGGCGACTTCGT |
| 301 ¤ | 04042-L03755 | **BRCA2** | NM_000059.3; 130-131; 852 nt before ATG | 13-031.787718 | CGGGAGAAGCGTGAGGGGACAGATTTGTG-CCGGCGCGGTTTTTGTCAGCTTACTCCGGCCAAAAAAGA |
| 418 | 01617-L01199 | **BRCA2** | NM_000059.3; 9100-9101 | 13-031.851549 | GGCCATGGAATCTGCTGAACAAAA-GGAACAAGGTTTATCAAGGGATGTCACAACCGTGTGGAAGT TGCG |
| 202 | 01245-L00793 | **MLH3** | NM_001040108.1; 3553-3554 | 14-074.578837 | GCGACCTTGTTCTTCCTTTCCTTCCGA-GAGCTCGAGCAGAGAGGACTGTGATGAGACAGGATAACAG |
| 281 « | 01832-L01397 | **TSC2** | NM_000548.3; 2080-2081 | 16-002.061787 | GAGCCAGAGAGAGGCTCTGAGAAGAAG-ACCAGCGGCCCCCTTTCTCCTCCCACAGGGCCTCCTG |
| 337 | 02416-L01862 | **CDH1** | NM_004360.3; 2654-2655 | 16-067.424756 | CTATGAAGGAAGCGGTTCCGAAGCTGCTA-GTCTGAGCTCCCTGAACTCCTCAGAGTCAGACAAAGACCAG GAC |
| 436 ¤ | 07946-L07727 | **CDH13** | NM_001257.3; 165-166; 42 nt after ATG | 16-081.218220 | TTCTGTGCGTTCTCCTGTCCCAG-GTAGGGAAGAGGGGCTGCCGGGCGCGCTCTG |
| 220 « | 03804-L00949 | **HIC1** | NM_006497.3, 11 nt before exon 1 | 17-001.905108 | CCGCTCCAGATAAGAGTGTGCGGA-AAGCGCGGCGGGGCTGAGACGCGACCAGGAC |
| 246 ¤ | 05162-L04543 | **BRCA1** | NM_007294.3; 178-179 | 17-038.530812 | TTCTCAGATAACTGGGCCCCTGC-GCTCAGGAGGCCTTCACCCTCTGCTCTGGGTAAAGG |
| 256 | 00587-L00382 | **BCL2** | NM_000633.2; 1151-1152 | 18-058.946868 | CTTCTCCTGGCTGTCTCTGAAGACTC-TGCTCAGTTTGGCCCTGGTGGGAGCTTG |
| 390 | 00713-L00108 | **KLK3** | NM_001648.2; 53-54 | 19-056.050015 | TGTGTCACCATGTGGGTCCCG-GTTGTCTTCCTCACCCTGTCCGTGACGTGGA |
| 142 | 02255-L03752 | **TIMP3** | NM_000362.4; 1015-1016; 172 nt before ATG | 22-031.527796 | TCCAGCGCCGAGGCAGCCTCGC-TGCGCCCCATCCCGTCCCGCCGGGCACTCGG |

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

« 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.

‡ Target sequence of this probe contains SNP rs104894994 (C/T) in the GCGC site, 6 nt right from the ligation site. This validated SNP with an allele frequency of 0.068%, when T-allele present, will inhibit the HhaI restriction, resulting in a false-positive methylation signal.

∫ Target sequence of this probe contains SNP rs3087462 (C/T) in the GCGC site, 3 nt right from the ligation site. This validated SNP with an allele frequency of 2.8%, when T-allele present, will inhibit the HhaI restriction, resulting in a false-positive methylation signal.

Λ Please treat positive signals indicating methylation of only the 211 nt CDKN2B probe with caution. This probe is incompletely digested when insufficient HhaI activity is present, e.g. in less pure samples, resulting in false positive results.

¤ These genes have a second probe in MLPA probemix ME002 Tumour suppressor mix 2, recognising a different CpG site.

+ More methylation probes for MLH1 are available in SALSA MLPA probemix ME011 MMR. The 167 nt probe is located in the Deng D-region. The 463 nt probe is located in the Deng B-region (Deng et al., 1999 *Cancer Research* 59; 2029-33).

» The 161 nt CDKN2A probe detects a sequence in front of the P14–INK4A-ARF promoter of CDKN2A instead of the ordinary CDKN2A P16 promoter. More probes for CDKN2A and CDKN2B are available in SALSA MLPA probemix ME024 9p21 CDKN2A/CDKN2B region.

° Probe renamed (was LOC254312).

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

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

# SALSA MLPA probemix ME001-C2 Tumour suppressor mix 1 sample pictures

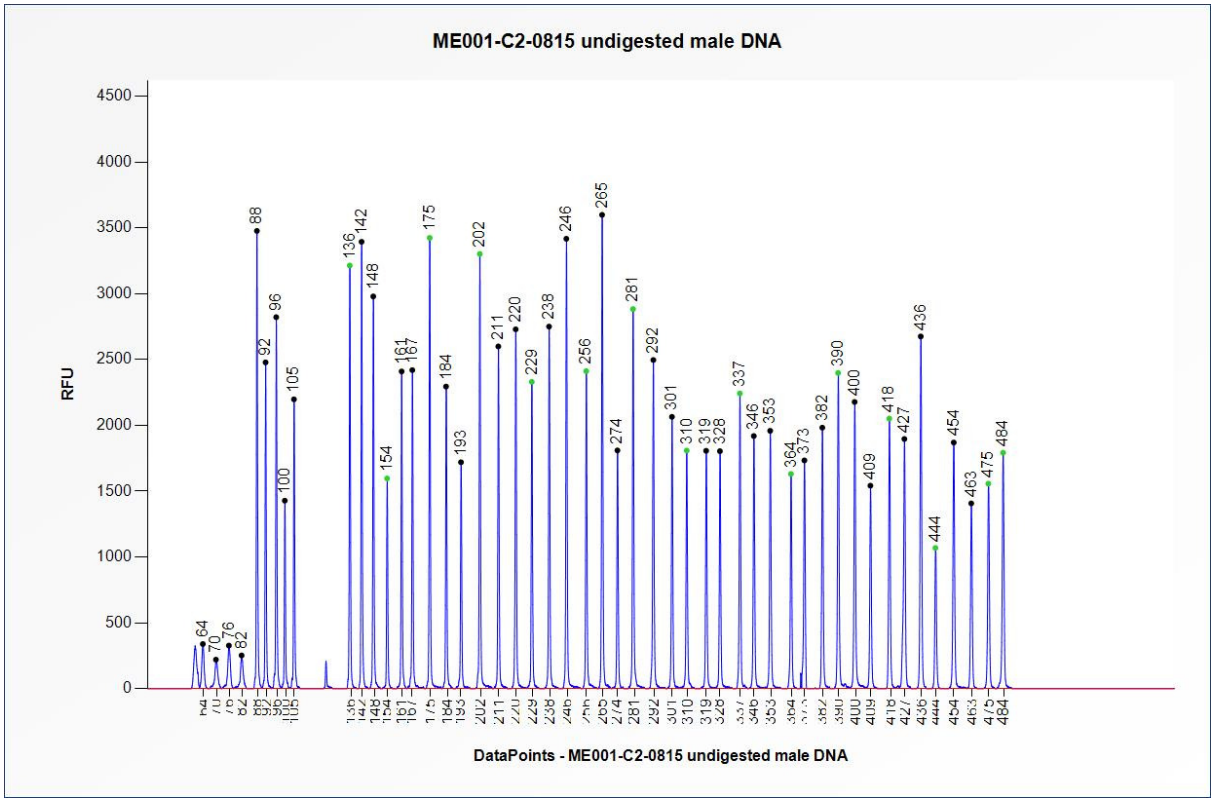


**Figure 1.** Capillary electrophoresis pattern of a sample of approximately 50 ng <u>undigested</u> human male control DNA analysed with SALSA MLPA probemix ME001-C2 Tumour suppressor mix 1 (lot C2-0815) for the quantification of copy numbers. Note: a non-specific peak is present at ~121 nt. This peak does not correspond to an MLPA probe.

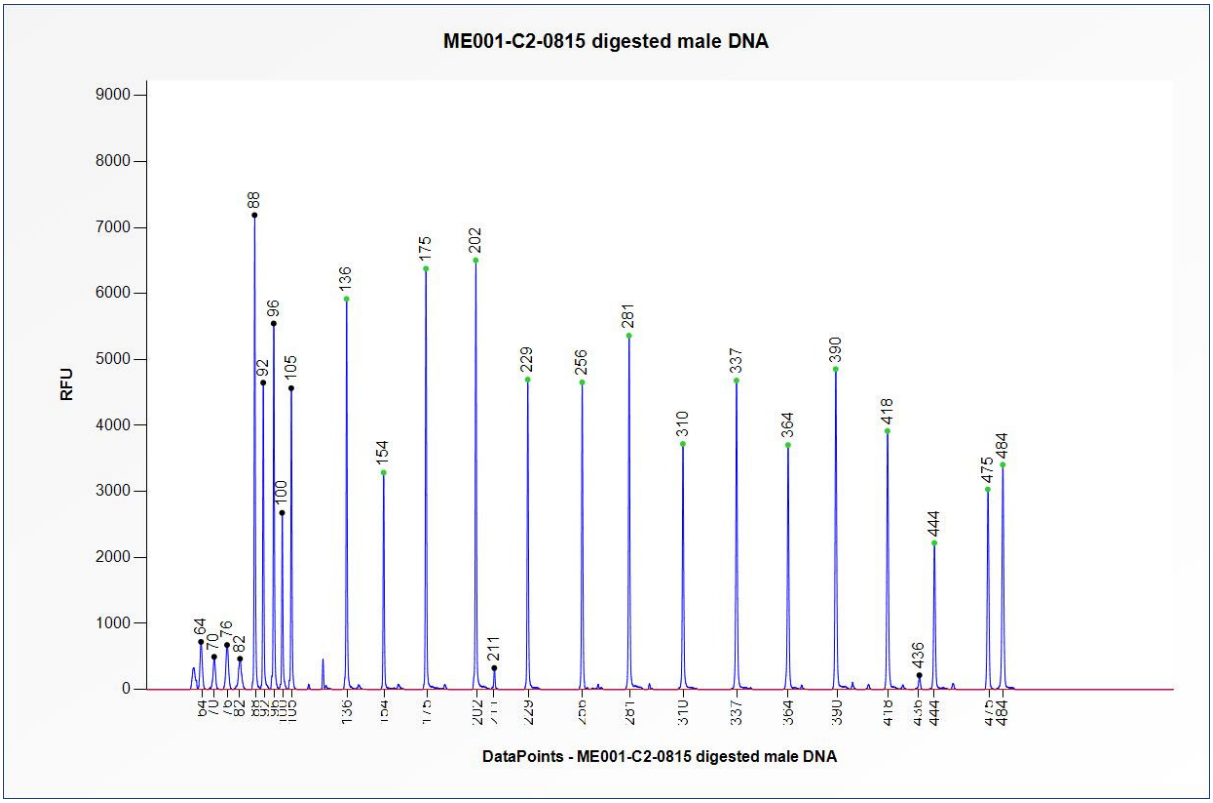


**Figure 2.** Capillary electrophoresis pattern of a sample of approximately 50 ng digested human male control DNA analysed with SALSA MLPA probemix ME001-C2 Tumour suppressor mix 1 (lot C2-0815) to determine the methylation status. Note: a non-specific peak is present at ~121 nt. This peak does not correspond to an MLPA probe.

| **Implemented Changes – compared to the previous product description version(s).** |
|---|
| *Version 17 – 07 December 2016 (16)*<br>- Warning regarding HhaI enzymes that are resistant to heat inactivation added under Methylation-specific MLPA section.<br>*Version 16 – 28 November 2016 (15)*<br>- Minor modification of the probemix name.<br>- Related SALSA MLPA probemixes and references added on page 2.<br>- Two probes renamed in Table 1 and Table 2.<br>- Footnotes added underneath Table 1 and Table 2.<br>- Column about the % of expected signal reduction added in Table 1.<br>- Ligation site and MV location adjusted for several probes in Table 2.<br>- Figure numbering modified on page 8.<br>- Various minor textual modifications.<br>*Version 15 (14)*<br>- Information in Table 1 and 2 corrected for the 292 nt probe 02203-L08261 as this probe is located in KLLN (transcript NM_001126049.1) upstream of PTEN.<br>- Related SALSA MLPA probemixes on page 2 modified.<br>- Two new references added on page 2.<br>*Version 14 (13)*<br>- Various textual and lay-out changes.<br>*Version 13 (09)*<br>- Warning added in Table 1 and 2, 220 nt probe 03804-L00949, 238 nt probe 03813-L03753, 281 nt probe 01832-L01397, 400 nt probe 04050-L01263, and 463 nt probe 02260-L01747.<br>*Version 12 (48)*<br>- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. |

*Version 11 (48)*
- Various minor textual changes.
- Warning added about a non-specific peak in the no DNA control.

*Version 08 (06)*
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Inclusion of probe ligation sites according to NM_ mRNA reference sequences or relative to the ATG translation start site in Table 2.