Product Description SALSA® MLPA® Probemixes P251-C2 NB mix 1 & P252-D1 NB mix 2 & P253-D1 NB mix 3

To be used with the MLPA General Protocol.

P251 version C2. As compared to version C1, three new reference probes have been added and two have been replaced, and multiple probes have a change in their length but not in the sequence detected.
P252 version D1. As compared to version C1, five new reference probes have been added and two have been replaced, and multiple probes have a change in their length but not in the sequence detected.
P253 version D1. As compared to version C1, four new reference probes have been added and two have been replaced, and multiple probes have a change in their length but not in the sequence detected.

For complete product history see page 12.

Catalogue numbers:
- **P251/P252/P253-025R:** SALSA MLPA Probemix P251/P252/P253 Neuroblastoma, 25 reactions.
- **P251/P252/P253-034R:** SALSA MLPA Probemix P251/P252/P253 Neuroblastoma, 34 reactions.
- **P251/P252/P253-050R:** SALSA MLPA Probemix P251/P252/P253 Neuroblastoma, 50 reactions.
- **P251/P252/P253-100R:** SALSA MLPA Probemix P251/P252/P253 Neuroblastoma, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General Information: The SALSA MLPA Probemixes P251-P252-P253 Neuroblastoma mix 1-3 are a **research use only (RUO)** assays for the detection of copy number changes of several chromosomal regions that frequently show copy number changes in neuroblastoma tumours.

Neuroblastoma (NB) is a relatively common paediatric cancer that usually occurs sporadically and frequently originates from one of the adrenal glands. Neuroblastoma is characterized by striking clinical heterogeneity, including cases that show spontaneous tumour regression. Neuroblastoma accounts for 6-8% of all cancers in children. Several acquired genetic alterations such as amplification of the *MYCN* oncogene, deletions of chromosome bands 1p36 and 11q23 and unbalanced gains of 17q regions have been well-characterized and show correlation with tumour behaviour, including response to treatment.

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.

Probemix content: The P251 probemix contains 36 probes in total for chromosomes 1, 3 and 11. The P252 probemix contains 34 probes in total for chromosomes 2 (*MYCN* region) and 17, and the P253 probemix contains 33 probes in total for chromosomes 4, 7, 9, 12 and 14. In addition, 13 reference probes are included in P251, 15 reference probes are included in P252 and 14 reference probes are included in P253, which all detect different autosomal chromosomal locations which are relatively stable in their copy number in neuroblastoma. However, it should be noted that neuroblastoma karyotypes can harbour multiple numerical and structural aberrations, which can complicate interpretation of these reference probes. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

The P251 probemix contains probes for chromosomes 1, 3 and 11:

- **1p36**: A deletion of the 1p36 region is present in 20-40% of NB patients with near-diploid/tetraploid tumours and is often associated with *MYCN* amplification (in 60% of cases). A probe for 1p36 tumour suppressor gene *CHD5* is included.
- **3p21-p22**: Deletions on the 3p arm have been described in neuroblastomas, and the *RASSF1A* gene is a candidate tumour suppressor gene in this region. The presence of 3p deletions appears to correlate with higher age at diagnosis.
- **11q**: Deletions of the 11q arm, and in particular 11q23, are common in NB patient samples and associated with higher a disease stage and poor prognosis.

The P252 probemix contains probes for chromosomes 2 and 17:

- **2p24**: Amplification of the proto-oncogene *MYCN* is found in 20-30% of all neuroblastomas. *MYCN* amplified tumours follow a very aggressive course and are associated with additional structural abnormalities—in particular with loss of 1p, gain of 17q and near-triploidy or -tetraploidy. *MYCN* amplification is often used for identification of high-risk patients. Additional probes for the nearby *NBAS*, *DDX1* and *ALK* genes are included.
- **2q33**: Loss of 2q33 has been reported in neuroblastomas and has been associated with loss of expression of *CASP8*.
- **17p**: Gains of the 17p probes together with gains of the 17q probes would indicate complete chromosome 17 gains, in contrast to the frequent unbalanced 17q gains that are often associated with translocations. Three probes for *TP53* have been included but *TP53* mutations and deletions might be rare in neuroblastomas.
- **17q**: Unbalanced gain of 17q is present in approximately 50% of patients and is associated with a poor outcome in neuroblastomas. It often results from an unbalanced translocation with 1p or 11q. Gain of 17q, in unbalanced translocations or as part of whole chromosome gain, is seen in 80% of neuroblastomas. Whole chromosome 17 gain is typically seen in near-triploid tumours with favourable prognosis. Please note that triploidy of all chromosomes cannot be detected by MLPA as only *relative* gains or losses are detected.

The P253 probemix contains probes for chromosomes 4, 7, 9, 12 and 14:

- Partial copy number changes of chromosomes 4, 7, 9, 12 and 14 have been described in neuroblastomas. Probes for *CDKN2A* and *PTPRD* on chromosome 9 have been included as *CDKN2A* is deleted (often homozygously) in many cancer types. The *PTPRD* gene has also been identified as a candidate tumour suppressor gene in neuroblastoma.

These probemixes contains nine quality control fragments generating amplification products between 64 and 121 nt: four DNA Quantity Fragments (Q-fragments), two DNA Denaturation Fragments (D-fragments), one benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)
88-96	D-fragments (Low signal of 88 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

P252 competitor mix information

Samples with very high levels of *MYCN* amplification exhibit very high signals for the *MYCN* probes and low signals for other probes, making it difficult to analyse the latter. Therefore, the P252 probemix is shipped together with a vial of the **P252 competitor mix**. When a sample shows a very high level of *MYCN* amplification it can be retested with the competitor mix. This competitor mix contains oligonucleotides that can be included at the start of the MLPA reaction. Adding the competitors specifically reduces the signal of the eight *MYCN* region probes, making it possible to examine changes in other genes/chromosomal areas.

Instructions for use:
Denature 4 µl sample DNA by heating 5 minutes at 98°C.
Add 1.5 µl MLPA Buffer + 1.5 µl P252 probemix + 1 µl of P252 competitor mix.
Proceed with the MLPA protocol starting with one minute incubation at 95°C and 16 hour incubation at 60°C followed by the ligation and PCR steps.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola, 2012.

Required specimens: Extracted DNA, which includes DNA derived from paraffin-embedded tissues, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from healthy individuals without a history of cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments.

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of all probes in the reference samples should be <0.10. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.

- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the majority of genetic alterations in the genes and chromosomal regions included in these probemixes are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemixes P251-P252-P253 Neuroblastoma.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- 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. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

COSMIC mutation database: http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC database. Recommendations for the nomenclature to describe deletions/duplications can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNPs and unusual results to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P251-C2 Neuroblastoma mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)			
		Reference	Chr. 1	Chr. 3	Chr. 11
64-105	Control fragments – see table in probemix content section for more information				
125 *	Reference probe 21195-L25924	21q22			
130 ¤	**PDE4DIP probe** 05712-L05712		1**q**21		
138 *	Reference probe 00797-L30622	5q31			
142	**ST5 probe** 06679-L06257				11p15
149 ¥	**HMBS probe** 01662-L30621				11**q**23
155	Reference probe 04566-L03955	16q13			
160	**PLPP3 probe** 02876-L02343		1p32		
166 «	**GABRD probe** 04690-L07966		1p36		
172	**GSTP1 probe** 06819-L07011				11**q**13
178	Reference probe 04858-L04242	5p13			
184	**CASP1 probe** 00559-L00128				11**q**22
190	**PTPRJ probe** 05918-L05363				11p11
196	**TGFBR2 probe** 03861-L03610			3p24	
202 +	Reference probe 10697-L12697	6p12			
211	**PIK3CA probe** 03826-L03222			3**q**26	
220	**KIF1B probe** 04682-L04060		1p36		
226	**LMO1 probe** 16709-L19293				11p15
232	**LMO1 probe** 16712-L19296				11p15
238	**CADM1 probe** 01640-L01178				11**q**23
247	Reference probe 07695-L07419	21q22			
254 ¥ «	**ABCC8 probe** 21876-L30842				11p15
260 *	Reference probe 12432-L30843	22q12			
266 ¥	**CD44 probe** 02245-L30511				11p13
274	**LHX4 probe** 07233-L06883		1**q**25		
283	**ROBO2 probe** 06447-L05973			3p12	
292	Reference probe 04224-L03560	19q13			
301	**CNTN5 probe** 08313-L08182				11**q**22
311 +	Reference probe 06425-L05951	6p22			
320	**LIN9 probe** 12058-L03618		1**q**42		
327 «	**TP73 probe** 01682-L01262		1p36		
337	**ATM probe** 02664-L02131				11**q**22
346 *	Reference probe 16440-L30623	18q21			
355	**PTAFR probe** 02267-L01425		1p35		
364	**SEMA3B probe** 03210-L02625			3p21	
373	Reference probe 03919-L03374	15q21			
382 ¥	**AKT3 probe** 21295-L30115		1**q**44		
396 ¥	**ZIC1 probe** 08544-L30513			3**q**24	
404 ¥	**RASSF1 probe** 03991-L30512			3p21	
409	**THY1 probe** 04777-L04125				11**q**23
418	**VHL probe** 01161-L00717			3p25	
427	**KMT2A probe** 01637-L01175				11**q**23
436	**PARK7 probe** 02188-L01686		1p36		
445	**ZMYND10 probe** 03207-L02622			3p21	
454	**CTNNB1 probe** 00673-L00117			3p22	
463	**NTNG1 probe** 16354-L06009		1p13		
475	Reference probe 12066-L13192	20q13			
484	**CASR probe** 02683-L02148			3**q**21	
496 ¥ «	**CHD5 probe** 09114-L25958		1p36		
504 *	Reference probe 21229-L30802	10p11			

* New in version C2 (from lot C2-0618 onwards).
¥ Changed in version C2 (from lot C2-0618 onwards). Small change in length, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
+ A recent article by Costa and Seuánez, 2018, reports that the 6p region can be gained in neuroblastoma samples. These two 6p reference probes can be removed from the normalisation calculations when necessary by adjusting the Coffalyser sheet.
¤ This probe detects a second target site on 1p11.2 (present in the hg38 genome build but not in the hg18/hg19 builds). The result of this probe should be disregarded if it differs from the results of other 1q probes.
Note: The identity of the genes detected by the reference probes is available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 1b. SALSA MLPA Probemix P252-D1 Neuroblastoma mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		Reference	Chr. 2	Chr. 17
64-105	Control fragments – see table in probemix content section for more information			
125 *	Reference probe 21195-L25924	21q22		
131 ¥	**TMEM18 probe** 06296-L25684		2p25	
136	**TP53 probe** 08304-L01158			17p13
142 «	**CFLAR probe** 00663-L00074		2**q**33	
148 *	Reference probe 05170-L21820	13q12		
155	Reference probe 04566-L03955	16q13		
160	**TBCD probe** 08306-L01293			17**q**25
168 ¥	**NF1 probe** 02514-L30629			17**q**11
173 ¥	**SGCA probe** 03373-L30630			17**q**21
178	Reference probe 04858-L04242	5p13		
184	**ERBB2 probe** 00991-L00146			17**q**12
190	**NBAS probe** 08317-L08186		2p24	
195	**BMPR2 probe** 12059-L09026		2**q**33	
202 +	Reference probe 10697-L12697	6p12		
213 ¥	**NBAS probe** 21789-L30625		2p24	
220	**RECQL5 probe** 04170-L03525			17**q**25
226 ¥	**WSB1 probe** 05736-L31080			17**q**11
232 ¥	**PAFAH1B1 probe** 04605-L30632			17p13
239	**SCN1A probe** 04543-L03932		2**q**24	
247	Reference probe 07695-L07419	21q22		
257	**TOP2A probe** 01055-L00628			17**q**21
265	**CASP8 probe** 02761-L02210		2**q**33	
274	**DDX1 probe** 08319-L08188		2p24	
283	**RPIA probe** 05713-L05151		2p11	
292	Reference probe 04224-L03560	19q13		
301 «	**BMPR2 probe** 04013-L03436		2**q**33	
311 +	Reference probe 06425-L05951	6p22		
319	**DDX1 probe** 08320-L08189		2p24	
333 ¥	**ALK probe** 08322-L30633		2p23	
339 ¥	**TPO probe** 11049-L30634		2p25	
346 *	Reference probe 16440-L30623	18q21		
353 «	**MYCN probe** 12060-L09025		2p24	
361 *	Reference probe 10086-L20983	8q22		
370 ¥	**WSB1 probe** 08326-L22797			17**q**11
378 ¥	Reference probe 03919-L30636	15q21		
384	**BIRC5 probe** 03025-L02411			17**q**25
391	**TOB1 probe** 04778-L04126			17**q**21
400	**SECTM1 probe** 01088-L00647			17**q**25
409	**RTN4 probe** 00963-L00550		2p16	
420	**DYSF probe** 08839-L13359		2p13	
429 *	Reference probe 12456-L23201	22q12		
436 «	**MYCN probe** 03327-L02466		2p24	
445	**WSB1 probe** 08328-L09024			17**q**11
454	**TP53 probe** 08785-L01159			17p13
465	**TP53 probe** 00844-L06726			17p13
475	Reference probe 12066-L13192	20q13		
486 ¥	**ALK probe** 15397-L30899		2p23	
493 *	Reference probe 14909-L27536	18p11		
500 *	Reference probe 21229-L29604	10p11		

* New in version D1 (from lot D1-0618 onwards).
¥ Changed in version D1 (from lot D1-0618 onwards). Small change in length, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
+ A recent article by Costa and Seuánez, 2018, reports that the 6p region can be gained in neuroblastoma samples. These two 6p reference probes can be removed from the normalisation calculations when necessary by adjusting the Coffalyser sheet.
Note: The identity of the genes detected by the reference probes is available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 1c. SALSA MLPA Probemix P253-D1 Neuroblastoma mix 3

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)					
		Reference	Chr. 4	Chr. 7	Chr. 9	Chr. 12	Chr. 14
64-105	Control fragments – see table in probemix content section for more information						
125 *	Reference probe 21195-L25924	21q22					
136 «	**SHH probe** 06358-L05874			7**q**36			
142	**PKP2 probe** 12061-L04788	12p11					
148 ¥	**SPON2 probe** 21907-L30514		4p16				
155	Reference probe 04566-L03955	16q13					
160 ¥	**TGFB3 probe** 21908-L31105						14**q**24
166	**ATL1 probe** 05279-L04660						14**q**22
172	**PTPRD probe** 08332-L08201				9p24		
178	Reference probe 04858-L04242	5p13					
184	**TBX5 probe** 05687-L05129					12**q**24	
189 ¥ «	**IMPDH1 probe** 21909-L31106			7**q**32			
196	**GNRHR probe** 12062-L04183		4**q**13				
202 +	Reference probe 10697-L12697	6p12					
213 ¥	**TJP2 probe** 21910-L30680				9**q**21		
220	**COL2A1 probe** 07405-L07052					12**q**13	
232 ¥ «	**WFS1 probe** 05376-L30681		4p16				
240 ¥	**NFKBIA probe** 13706-L31107						14**q**13
247	Reference probe 07695-L07419	21q22					
254 ¥ «	**KCNIP4 probe** 21878-L16046		4p15				
265 ๑	**CDKN2A probe** 02238-L13510				9p21		
274	**CDKN2A probe** 01291-L00835				9p21		
283	**ERC1 probe** 06682-L06260					12p13	
292	Reference probe 04224-L03560	19q13					
303 ¥	**EGFR probe** 05961-L20432			7p11			
311 +	Reference probe 06425-L05951	6p22					
322 ¥	**CDKN1B** probe 02256-L30516					12p13	
329 ¥	**GLRB probe** 08956-L30517		4**q**32				
339 ¥	**MDM2 probe** 02894-L20364					12**q**15	
346 *	Reference probe 16440-L30623	18q21					
352 *	Reference probe 11653-L22884	5q32					
360 ¥ «	**ELN probe** 12063-L22813			7**q**11			
368 ¥	**OCIAD1 probe** 12064-L31255		4p11				
373	Reference probe 03919-L03374	15q21					
382	**KLKB1 probe** 01136-L00694		4**q**35				
391 «	**MOAP1 probe** 00947-L01595						14**q**32
400 *	Reference probe 10091-L10515	8q22					
408 ¥	**KRIT1 probe** 04349-L31108			7**q**21			
413 ¥	**TGFBR1 probe** 04653-L31256				9**q**22		
421	**GHRHR probe** 07215-L13361			7p14			
427	**TSC1 probe** 04796-L04171				9**q**34		
436	**POMT1 probe** 04129-L03486				9**q**34		
445	**IL2 probe** 00627-L00183		4**q**27				
454	**DNAI1 probe** 08059-L07840				9p13		
468 ¥	**PTPRD probe** 08330-L30682				9p23		
480 ¥	Reference probe 12066-L31109	20q13					
490 *	Reference probe 12461-L21828	22q12					
500 *	Reference probe 21229-L29604	10p11					

* New in version D1 (from lot D1-0618 onwards).

¥ Changed in version D1 (from lot D1-0618 onwards). Small change in length, no change in sequence detected.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

๑ In several patients from the Netherlands and Belgium, a 6 bp deletion (GTACGC) has been reported in the target sequence of this CDKN2A probe (02238-L13510; 265 nt). However, the pathological significance of this deletion is unclear.

\+ A recent article by Costa and Seuánez, 2018, reports that the 6p region can be gained in neuroblastoma samples. These two 6p reference probes can be removed from the normalisation calculations when necessary by adjusting the Coffalyser sheet.

Note: The identity of the genes detected by the reference probes is available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 2a. P251-C2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
Chromosome 1						
166 «	04690-L07966	*GABRD*	1p36	CGGCGACTACGT-GGGCTCCAACCT	01-001.946	1.6 Mb
327 «	01682-L01262	*TP73*	1p36	GAGACCCGGGTG-TCAGGAAAGATG	01-003.558	2.6 Mb
496 «	09114-L25958	*CHD5*	1p36	GTTTCTTCTTCT-TAGGAAGGCTCA	01-006.151	1.8 Mb
436	02188-L01686	*PARK7*	1p36	ATGGCGGCTATC-AGGCCCTTCCGG	01-007.954	2.4 Mb
220	04682-L04060	*KIF1B*	1p36	CGTGGGGTCCTT-TTGCAGGCCCTC	01-010.358	18.0 Mb
355	02267-L01425	*PTAFR*	1p35	CATCTTCATCGT-GTTCAGCTTCTT	01-028.350	28.5 Mb
160	02876-L02343	*PLPP3*	1p32	CCCCTTGGACTT-TAGAACGATTTA	01-056.817	50.9 Mb
463	16354-L06009	*NTNG1*	1p13	GGATAAGGCTGT-TAAGACCAGCCG	01-107.669	36.0 Mb
130 ¤	05712-L05712	*PDE4DIP*	1**q**21	GCTACATCTGTT-GGAGGAGCCAAC	01-143.658	34.8 Mb
274	07233-L06883	*LHX4*	1**q**25	CATGGCCCCGCA-TGGTCCCCTCTC	01-178.502	46.0 Mb
320	12058-L03618	*LIN9*	1**q**42	GGCCTTCTCGAT-TTTTTATGACCC	01-224.521	17.4 Mb
382	21295-L30115	*AKT3*	1**q**44	TTGCCTCTGCAG-TCTGTCTGCTAC	01-241.876	-
Chromosome 3						
418 #	01161-L00717	*VHL*	3p25	CTAGTCAAGCCT-GAGAATTACAGG	03-010.166	20.494.8 Mb
196	03861-L03610	*TGFBR2*	3p24	CTGTGACAACCA-GAAATCCTGCAT	03-030.661	10579.7 Mb
454	00673-L00117	*CTNNB1*	3p22	GGCCATGGAACC-AGACAGAAAAGC	03-041.241	9041.6 Mb
364	03210-L02625	*SEMA3B*	3p21	ACCTGGACAACA-TCAGCAAGCGGG	03-050.283	60.4 **k**b
404	03991-L30512	*RASSF1*	3p21	TCCTGCAGAAGT-ACTCCTATTGCC	03-050.343	12.8 **k**b
445	03207-L02622	*ZMYND10*	3p21	AAGACACTGTCT-TGGACTTGGTAG	03-050.356	26874.3 Mb
283	06447-L05973	*ROBO2*	3p12	GGAAGCTACGTT-TGTGTTGCGAGG	03-077.230	46255.1 Mb
484	02683-L02148	*CASR*	3**q**21	GCCCAGATGACT-TCTGGTCCAATG	03-123.485	25130.9 Mb
396	08544-L30513	*ZIC1*	3**q**24	ATGCACTCTATG-TGTTCAGGAAGC	03-148.616	31814.4 Mb
211	03826-L03222	*PIK3CA*	3**q**26	ACACGTTCATGT-GCTGGATACTGT	03-180.430	-
Chromosome 11						
232	16712-L19296	*LMO1*	11p15	GCCACATTAGAA-CTTCTCCGTCCT	11-008.203	39.0 **k**b
226	16709-L19293	*LMO1*	11p15	TTCACTCCTGAA-TGTAATTCTAGC	11-008.242	0.5 Mb
142	06679-L06257	*ST5*	11p15	GCCACCACTAGT-ACCATGAGTCCC	11-008.789	8.6 Mb
254 «	21876-L30842	*ABCC8*	11p15	CACTTCCAGATT-TAACCTGGACCC	11-017.373	17.7 Mb
266	02245-L30511	*CD44*	11p13	CCCGCGCCCTCC-GTTCGCTCCGGA	11-035.117	13.0 Mb
190	05918-L05363	*PTPRJ*	11p11	GGGGAGACAGAT-TCTTCCAATCTC	11-048.106	19.0 Mb
172	06819-L07011	*GSTP1*	11**q**13	ACCAGTCCAATA-CCATCCTGCGTC	11-067.109	32.6 Mb
301	08313-L08182	*CNTN5*	11**q**22	ATTCTTGTTGCA-TGGAAACACATT	11-099.684	4.7 Mb
184 #	00559-L00128	*CASP1*	11**q**22	CCGCACACGTCT-TGCTCTCATTAT	11-104.406	3.3 Mb
337	02664-L02131	*ATM*	11**q**22	TTTTTCCGATGC-TGTTTGGATAAA	11-107.684	7.2 Mb
238	01640-L01178	*CADM1*	11**q**23	GATCCGGGGAAA-GCAAAACCCGAA	11-114.881	3.0 Mb
427	01637-L01175	*KMT2A*	11**q**23	GGACCCCGGATT-AAACATGTCTGC	11-117.853	0.6 Mb
149	01662-L30621	*HMBS*	11**q**23	CATCTCTATAGA-GTGGACCTGGTT	11-118.466	0.3 Mb
409	04777-L04125	*THY1*	11**q**23	GGCTGTCTTTTT-GTACTTTTTGTT	11-118.795	-

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

¤ This probe detects a second target site on 1p11.2 (present in the hg38 genome build but not in the hg18/hg19 builds). The result of this probe should be disregarded if it differs from the results of other 1q probes.

Note: Please notify us of any mistakes: info@mlpa.com.

Table 2b. P252-D1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
Chromosome 2						
131	06296-L25684	*TMEM18*	2p25	TCCTCACCTGCT-TGTCCTCCCGAA	02-000.666	0.8 Mb
339	11049-L30634	*TPO*	2p25	GATGACCGCTAT-TCTGACCTCCTG	02-001.439	13.8 Mb
190	08317-L08186	***NBAS***	2p24	GTCCCTCCTGCT-TCCATCTCTGAA	02-015.237	0.2 Mb
213	21789-L30625	***NBAS***	2p24	CTGGTTCTCTGT-GACAATTTGGTT	02-015.475	0.2 Mb
274	08319-L08188	***DDX1***	2p24	TCAAAGCAGAGA-AGTAAAGGAATG	02-015.660	14.7 **k**b
319	08320-L08189	***DDX1***	2p24	GTGTCAACTGGA-AAGCTGAACTTA	02-015.675	0.3 Mb
353 «	12060-L09025	***MYCN***	2p24	CTGTCACCACAT-TCACCATCACTG	02-016.003	0.2 **k**b
436 «	03327-L02466	***MYCN***	2p24	TGCACCCCCACA-GAAGAAGATAAA	02-016.003	13.3 Mb
486	15397-L30899	***ALK***	2p23	TTTCTCTTGGAT-ATATGCCATACC	02-029.274	0.5 Mb
333	08322-L30633	***ALK***	2p23	ATCTCACCTGGA-TAATGAAAGACT	02-029.794	25.3 Mb
409	00963-L00550	*RTN4*	2p16	CTGGAGAGACAT-TAAGAAGACTGG	02-055.068	16.7 Mb
420	08839-L13359	*DYSF*	2p13	TGCCATGAAGCT-GGTGAAGCCCTT	02-071.767	17.0 Mb
283 #	05713-L05151	*RPIA*	2p11	TGGTTCTACAAT-TGTCCATGCTGT	02-088.779	77.8 Mb
239	04543-L03932	*SCN1A*	2**q**24	ATAGGCCACATT-CAAAGGATGGAT	02-166.564	35.1 Mb
142 «	00663-L00074	*CFLAR*	2**q**33	TGTCTGTCGGGG-ACTTGGCTGAAC	02-201.703	0.1 Mb
265	02761-L02210	*CASP8*	2**q**33	TGTCCAGCGCTC-GGGCTTTAGTTT	02-201.831	1.1 Mb
195	12059-L09026	*BMPR2*	2**q**33	GGATTTGTTGTT-TTCGAAATCAGA	02-202.950	0.2 Mb
301 «	04013-L03436	*BMPR2*	2**q**33	TTGAGGATATGC-AGGTTCTCGTGT	02-203.115	-
Chromosome 17						
232 #	04605-L30632	*PAFAH1B1*	17p13	CTGTTCTGCAGA-TATGACCATTAA	17-002.520	5.0 Mb
454	08785-L01159	*TP53*	17p13	TTCCGAGAGCTG-AATGAGGCCTTG	17-007.515	3.1 **k**b
136	08304-L01158	*TP53*	17p13	CTGTCCTGGGAG-AGACCGGCGCAC	17-007.518	2.5 **k**b
465	00844-L06726	*TP53*	17p13	CATCTACAGTCC-CCCTTGCCGTCC	17-007.520	15.1 Mb
370	08326-L22797	*WSB1*	17**q**11	CTCTTCTCTGTT-GTTGGGTCCGCA	17-022.645	17.2 **k**b
226	05736-L31080	*WSB1*	17**q**11	ATTGATGAGGAT-TATCCAGTGCAA	17-022.663	0.7 **k**b
445	08328-L09024	*WSB1*	17**q**11	GTCGCATGTCAA-TCCGAAGAGTGA	17-022.663	3.9 Mb
168 #	02514-L30629	*NF1*	17**q**11	TCTTTCCTTCAT-AAGTGACGGCAA	17-026.612	8.5 Mb
184	00991-L00146	*ERBB2*	17**q**12	GGTGCAGGGCTA-CGTGCTCATCGC	17-035.118	0.7 Mb
257	01055-L00628	*TOP2A*	17**q**21	AAGCCCTTCAAT-GGAGAAGATTAT	17-035.823	9.8 Mb
173	03373-L30630	*SGCA*	17**q**21	CCATGTTCAATG-TGCACACAGGTG	17-045.608	0.7 Mb
391	04778-L04126	*TOB1*	17**q**21	TGTCAACATTTT-TGGTGAAGAACT	17-046.296	24.8 Mb
220	04170-L03525	*RECQL5*	17**q**25	GGCTGCAAATGT-TGTGGTCAAGTG	17-071.136	2.6 Mb
384	03025-L02411	*BIRC5*	17**q**25	GCATTCGTCCGG-TTGCGCTTTCCT	17-073.724	4.1 Mb
400	01088-L00647	*SECTM1*	17**q**25	TCTTCATCCTCT-TGGTCGCTCTGG	17-077.874	0.6 Mb
160	08306-L01293	*TBCD*	17**q**25	ACACGCAGCCAA-TGATAGACCACC	17-078.452	-

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

The signals of the *MYCN* region (2p23-p24) probes that are **indicated in bold** can be reduced by the inclusion of the P252 competitor mix during the PCR reaction.

Note: Please notify us of any mistakes: info@mlpa.com.

Table 2c. P253-D1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
Chromosome 4						
148	21907-L30514	*SPON2*	4p16	CTTCCCCAAGCA-GTACCCCCTGTT	04-001.156	5.2 Mb
232 «	05376-L30681	*WFS1*	4p16	CTCAATGCCACA-GCCTCGTTGGAG	04-006.330	15.2 Mb
254 «	21878-L16046	*KCNIP4*	4p15	GTGGAAAGCATT-TCGGCTCAGCTG	04-021.559	27.0 Mb
368	12064-L31255	*OCIAD1*	4p11	ATGCTTCCTCAT-TATGAGCCAATT	04-048.549	19.8 Mb
196	12062-L04183	*GNRHR*	4**q**13	TGGAACATTACA-GTCCAATGGTAT	04-068.302	55.3 Mb
445	00627-L00183	*IL2*	4**q**27	ACAATGTACAGG-ATGCAACTCCTG	04-123.597	34.7 Mb
329	08956-L30517	*GLRB*	4**q**32	TATTGCTTGCCT-TCTCTTTGGGTT	04-158.293	29.1 Mb
382	01136-L00694	*KLKB1*	4**q**35	ATGCCCAATACT-GCCAGATGAGGT	04-187.390	-
Chromosome 7						
421	07215-L13361	*GHRHR*	7p14	TTCCTCAACCAA-GAGGTGTGTGAT	07-030.983	24.2 Mb
303	05961-L20432	*EGFR*	7p11	TCATGGGAGAAA-ACAACACCCTGG	07-055.201	17.9 Mb
360 «	12063-L22813	*ELN*	7**q**11	ACCTCATCAACG-TTGGTGCTACTG	07-073.121	18.6 Mb
408	04349-L31108	*KRIT1*	7**q**21	CAATCCAAACCT-TTTAAATGGACA	07-091.694	36.1 Mb
189 « #	21909-L31106	*IMPDH1*	7**q**32	GGGGCCTCCGTA-GTGGCGGCCAGC	07-127.822	27.5 Mb
136	06358-L05874	*SHH*	7**q**36	CAAGGCACATAT-CCACTGCTCGGT	07-155.292	-
Chromosome 9						
172	08332-L08201	*PTPRD*	9p24	CACAAGGGAGCA-TCATACGTCTTC	09-008.476	1.5 Mb
468	08330-L30682	*PTPRD*	9p23	TAGAGGTGTCTG-ACTGACAGCATG	09-009.929	12.0 Mb
274	01291-L00835	*CDKN2A*	9p21	TGAAAGAACCAG-AGAGGCTCTGAG	09-021.958	27.3 **k**b
265 ๑	02238-L13510	*CDKN2A*	9p21	AGACCGGAGAGA-GAACGTACGCCG	09-021.985	12.5 Mb
454	08059-L07840	*DNAI1*	9p13	ACTGAAGTGGAA-GAGAGTCCAGAT	09-034.449	36.6 Mb
213	21910-L30680	*TJP2*	9**q**21	CGTTTTTTATAA-GAAGCCACTTTG	09-071.041	30.0 Mb
413	04653-L31256	*TGFBR1*	9**q**22	GATGGGTCAGAA-GGTACAAGATCA	09-100.950	32.4 Mb
436	04129-L03486	*POMT1*	9**q**34	GGAGCTCCACTT-TTCTCATTGTGC	09-133.373	1.4 Mb
427	04796-L04171	*TSC1*	9**q**34	ACCCAGCAAGTC-TGTCGACTGGAC	09-134.770	-
Chromosome 12						
283	06682-L06260	*ERC1*	12p13	GAACGGGACAAT-GCAGAACTGCAG	12-001.470	11.3 Mb
322	02256-L30516	*CDKN1B*	12p13	GACTCCGACGCC-GGCAAGGTTTGG	12-012.762	20.1 Mb
142 #	12061-L04788	*PKP2*	12p11	GAAGATGTGACG-GACTCATTGACT	12-032.868	13.8 Mb
220	07405-L07052	*COL2A1*	12**q**13	CACAGGGTCCTT-CTGGAGACCAAG	12-046.657	20.8 Mb
339	02894-L20364	*MDM2*	12**q**15	CGAGATCCTGCT-GCTTTCGCAGCC	12-067.488	45.8 Mb
184	05687-L05129	*TBX5*	12**q**24	GCCTGACGCAAA-AGACCTGCCCTG	12-113.326	-
Chromosome 14						
240	13706-L31107	*NFKBIA*	14**q**13	CTACCAGGGCTA-TTCTCCCTACCA	14-034.941	15.2 Mb
166	05279-L04660	*ATL1*	14**q**22	AGCCAGTGAAAA-AGGCAGGACCAG	14-050.124	25.4 Mb
160	21908-L31105	*TGFB3*	14**q**24	TGCACCCAGGAA-AACACCGAGTCG	14-075.517	17.2 Mb
391 «	00947-L01595	*MOAP1*	14**q**32	GTCTTGCAGGCT-GCTGGACCTCGG	14-092.720	-

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

๑ In several patients from the Netherlands and Belgium, a 6 bp deletion (GTACGC) has been reported in the target sequence of this *CDKN2A* probe (02238-L13510; 265 nt). However, the pathological significance of this deletion is unclear.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Note: Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

P088 Oligodendroglioma 1p-19q:	Contains probes for the 1p and 19q chromosomal arms, *CDKN2A & CDKN2B* genes and *IDH1* R132H/C and *IDH2* R172M/K point mutations.
P323 CDK4-HMGA2-MDM2:	Contains probes for the chromosome 12p and 12q arms.
P419 CDKN2A/2B-CDK4:	Contains probes for each exon of the *CDKN2A, CDKN2B* and *CDK4* genes.
ME024 9p21 region:	Contains probes for the detection of both copy number and methylation status of genes in the 9p21 (*CDKN2A* and *CDKN2B*) region.

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P251 Product history	
Version	*Modification*
C2	Three new reference probes have been added and two have been replaced, and multiple probes have a change in their length but not in the sequence detected.
C1	Two new LMO1 probes and new QDX fragments (QDX2) have been included.
B1	Several probes have been replaced and extra X- and Y-control fragments have been added. In addition, new reference probes have been included.
A1	First release.

P252 Product history	
Version	*Modification*
D1	Five new reference probes have been added and two have been replaced, and multiple probes have a change in their length but not in the sequence detected.
C1	One probe for 2p telomere and an extra ALK probe have been added.
B1	Several probes have been replaced and extra X- and Y-control fragments have been added. In addition, new reference probes have been included.
A1	First release.

P253 Product history	
Version	*Modification*
D1	Four new reference probes have been added and two have been replaced, and multiple probes have a change in their length but not in the sequence detected.
C1	One NFKBIA probe has been replaced.
B1	Several probes have been replaced and extra X- and Y-control fragments have been added. In addition, new reference probes have been included.
A1	First release.

Implemented changes in the product description

Version C2/D1-02 – 28 November 2018 (01P)
- Additional information about two target locations for PDE4DIP (probe 05712-L05712 at 130 nt) added to Table 1a and Table 2b.

Version C2/D1-01 – 27 July 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Warning added to Tables 2a-c for probe specificity relying on a single nucleotide difference between target gene and a related gene or pseudogene.

Version 17 – 13 January 2017 (T08)
- Warning added in Table 1b and Table 2b, 301 nt probe 04013-L03436, and in Table 1c and Table 2c, 189 nt probe 06989-L06591, 256 nt probe 03336-13509.

Version 16 – 15 April 2016 (T08)
- *PLPP3* gene name has been changed (*PPAP2B* previously) as we have adopted the novel HUGO name for this gene.

Version 15 – 07 January 2016 (T08)
- New reference added on page 2.

Version 14 – 28 October 2015 (T08)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new pictures included).
- New references added on page 2.

- Information about partial sequence of probe added in Tables 2a, 2b and 2c.
- Removed notes regarding exon numbering, as it is not applicable to this product description.

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