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.

Version P251-C2 / P252-D1 / P253-D1
For complete product history see page 14 and 15.

Catalogue numbers:
- **P251/P252/P253-025R:** SALSA MLPA Probemix P251/P252/P253 NB mix 1, 2 & 3, 25 reactions.
- **P251/P252/P253-050R:** SALSA MLPA Probemix P251/P252/P253 NB mix 1, 2 & 3, 50 reactions.
- **P251/P252/P253-100R:** SALSA MLPA Probemix P251/P252/P253 NB mix 1, 2 & 3, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

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

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 NB mix 1, 2 & 3 are 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 up to 10% of all paediatric cancers. 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. For review please see e.g. Ambros et al. (2009) and Ahmed et al. (2017).

These SALSA MLPA probemixes are not CE/FDA registered for use in diagnostic procedures. Purchase of these products includes a limited license for research purposes.

Gene structure and transcript variants:
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
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Probemix content
The SALSA MLPA Probemix P251-C2 NB mix 1 contains 49 probes, and this includes 36 probes in total for chromosomes 1, 3 and 11. The SALSA MLPA Probemix P252-D1 NB mix 2 contains 49 probes, and this includes 34 probes in total for chromosomes 2 (*MYCN* region) and 17. The SALSA MLPA Probemix P253-D1 NB mix 3 contains 47 probes and this includes 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 that target relatively copy number stable regions in various cancer types including 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.mrcholland.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 and the *PTPRD* gene has also been identified as a candidate tumour suppressor gene in neuroblastoma.

Each probemix contains nine quality control fragments generating amplification products between 64 and 105 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.mrcholland.com.

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

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

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. 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 different healthy individuals without a history of neuroblastoma. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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 CM002 (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:
1. Denature 4 µl sample DNA by heating 5 minutes at 98°C.
2. Add 1.5 µl MLPA Buffer + 1.5 µl P252 probemix + 1 µl of P252 competitor mix.
3. 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.

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers listed in the table below from the Coriell Institute have been tested with P251-C2, P252-D1 and P253-D1 probemixes at MRC Holland and can be used as positive control samples to detect copy number alterations mentioned below. The quality of cell lines can change; therefore samples should be validated before use.

Coriell sample name	Chromosomal position of CNA (hg18)*	Altered target genes in P251-C2 NB mix 1	Expected copy number alteration
NA22977	1p36.33	*GABRD*	Heterozygous deletion
NA18827	1p36.33	*GABRD*	Heterozygous deletion
NA22995	1p36.32-p36.33	*GABRD, TP73*	Heterozygous deletion
NA22991	1p36.32-p36.33	*GABRD, TP73*	Heterozygous deletion

NA50276	1p36.22-p36.31	*CHD5, PARK7, KIF1B*	Heterozygous deletion
NA17941	1q21.2-q44	*PDE4DIP, LHX4, LIN9, AKT3*	Heterozygous duplication
NA06038	1q25.2	*LHX4*	Heterozygous deletion
NA00214	1q25.2	*LHX4*	Heterozygous deletion
NA05347	1q42.12-q44	*LIN9, AKT3*	Heterozygous duplication
NA06473	1q44	*AKT3*	Heterozygous deletion
NA03503	3p25.3	*VHL*	Heterozygous duplication
NA10985	3p25.3	*VHL*	Heterozygous deletion
NA04127	3p21.31-p25.3	*VHL, TGFBR2, CTNNB1, SEMA3B, RASSF1, ZMYND10*	Heterozygous duplication
NA08778	3q21.1	*CASR*	Heterozygous deletion
NA03563	3q21.1-q26.32	*CASR, ZIC1, PIK3CA*	Heterozygous duplication
NA11428	3q24-q26.32	*ZIC1, PIK3CA*	Heterozygous duplication
NA20022	3q24-q26.32	*ZIC1, PIK3CA*	Heterozygous duplication
NA10175	3q26.32	*PIK3CA*	Heterozygous duplication
NA09709	11p13	*CD44*	Heterozygous deletion
NA22633	11p11.2	*PTPRJ*	Heterozygous deletion
NA00959	11q13.2-q23.3	*GSTP1, CNTN5, CASP1, ATM, CADM1, KMT2A, HMBS, THY1*	Heterozygous duplication
NA09596	11q22.1-q22.3	*CNTN5, CASP1, ATM*	Heterozygous deletion
NA15099	11q22.1-q23.3	*CNTN5, CASP1, ATM, CADM1, KMT2A, HMBS, THY1*	Heterozygous duplication
NA08618	11q22.1-q22.3	*CNTN5, CASP1, ATM*	Heterozygous duplication
Sample name	**Chromosomal position of CNA (hg18)***	**Altered target genes in P252-D1 NB mix 2**	**Expected copy number alteration**
NA00501	2p25.3	*TMEM18*	Heterozygous deletion
NA10401	2p25.3-q33.1	*TMEM18, TPO, NBAS, DDX1, MYCN, ALK, RTN4, DYSF, RPIA, SCN1A, CFLAR, CASP8, BMPR2*	Heterozygous duplication
NA04409	2p24.3-p25.3	*TMEM18, TPO, NBAS, DDX1, MYCN*	Heterozygous duplication
NA10951	2p25.3	*TPO*	Heterozygous duplication
NA00945	2p24.3	*NBAS, DDX1, MYCN*	Heterozygous deletion
NA09216	2p24.3	*NBAS, DDX1, MYCN*	Heterozygous deletion
NA10607	2q24.3	*SCN1A*	Heterozygous deletion
NA11213	2q33.1	*CFLAR, CASP8, BMPR2*	Heterozygous deletion
NA01229	2q33.1	*CFLAR, CASP8, BMPR2*	Heterozygous duplication
NA06047	17p13.3	*PAFAH1B1*	Heterozygous deletion
NA13031	17q21.33	*TOB1*	Heterozygous deletion
NA16445	17q25.3	*BIRC5, SECTM1, TBCD*	Heterozygous duplication
Sample name	**Chromosomal position of CNA (hg18)***	**Altered target genes in P253-D1 NB mix 3**	**Expected copy number alteration**
NA10947	4p15.31-p16.3	*SPON2, WSF1, KCNIP4*	Heterozygous duplication
NA03435	4p15.31-p16.3	*SPON2, WSF1, KCNIP4*	Heterozygous deletion
NA00782	4p13.2-q27	*GNRHR, IL2*	Heterozygous duplication
NA00501	4q27-q35.2	*IL2, GLRB, KLKB1*	Heterozygous duplication
NA10313	4q32.1-q35.2	*GLRB, KLKB1*	Heterozygous duplication
	7q36.3	*SHH*	Heterozygous deletion
NA03013	4q32.1-q35.2	*GLRB, KLKB1*	Heterozygous deletion
NA08763	7p15.1	*GHRHR*	Heterozygous deletion
NA07081	7p11.2-p15.1	*GHRHR, EGFR*	Heterozygous duplication
NA12590	7q11.23	*ELN*	Heterozygous deletion

NA10160	7q11.23-q21.2	*ELN, KRIT1*	Heterozygous deletion
NA12519	7q32.1	*IMPDH1*	Homozygous duplication
NA01220	7q36.3	*SHH*	Heterozygous duplication
NA10989	9p23-p24.1	*PTPRD*	Heterozygous deletion
NA01750	9p21.3-p24.1	*PTPRD, CDKN2A*	Heterozygous duplication
NA02819	9p13.3-p24.1	*PTPRD, CDKN2A, DNAI1*	Heterozygous duplication
NA13685	9q34.13	*POMT1, TSC1*	Heterozygous duplication
NA07981	12p11.21-p13.33	*ERC1, CDKN1B, PKP2*	Homozygous duplication
NA06801	14q13.2	*NFKBIA*	Heterozygous duplication
NA09888	14q22.1	*ALT*	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by the P251-C2/P252-D1/P253-D1 NB mix 1, 2 & 3 probemix.

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.mrcholland.com. The 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 each individual probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the final ratio (FR) of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

Please note that these above-mentioned final ratios are only valid for germline testing. Final ratios are affected both by the 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 subclonal cases.
- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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 (as described for *DMD* by 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.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P251/P252/P253 specific note

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood-derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- In most populations, most genetic alterations in gene/chromosomal region included in this probemix are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P251-C2, P252-D1 and P253-D1 NB mix 1, 2 & 3.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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, the 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, especially in solid tumours with more chaotic karyotypes.

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 of one or more exons can be found on http://varnomen.hgvs.org/.

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

Table 1. SALSA MLPA Probemix P251-C2 NB 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**21.1		
138	Reference probe 00797-L30622	5q31			
142 «	**DENND2B probe** 06679-L06257				11p15.4
149	**HMBS probe** 01662-L30621				11**q**23.3
155	Reference probe 04566-L03955	16q13			
160	**PLPP3 probe** 02876-L02343		1p32.2		
166 «	**GABRD probe** 04690-L07966		1p36.33		
172	**GSTP1 probe** 06819-L07011				11**q**13.2
178	Reference probe 04858-L04242	5p13			
184	**CASP1 probe** 00559-L00128				11**q**22.3
190	**PTPRJ probe** 05918-L05363				11p11.2
196	**TGFBR2 probe** 03861-L03610			3p24.1	
202 +	Reference probe 10697-L12697	6p12			
211	**PIK3CA probe** 03826-L03222			3**q**26.32	
220	**KIF1B probe** 04682-L04060		1p36.22		
226 «	**LMO1 probe** 16709-L19293				11p15.4
232	**LMO1 probe** 16712-L19296				11p15.4
238	**CADM1 probe** 01640-L01178				11**q**23.2
247	Reference probe 07695-L07419	21q22			
254 «	**ABCC8 probe** 21876-L30842				11p15.1
260	Reference probe 12432-L30843	22q12			
266 ±	**CD44 probe** 02245-L30511				11p13
274	**LHX4 probe** 07233-L06883		1**q**25.2		
283	**ROBO2 probe** 06447-L05973			3p12.3	
292	Reference probe 04224-L03560	19q13			
301	**CNTN5 probe** 08313-L08182				11**q**22.1
311 +	Reference probe 06425-L05951	6p22			
320	**LIN9 probe** 12058-L03618		1**q**42.12		
327 «	**TP73 probe** 01682-L01262		1p36.32		
337	**ATM probe** 02664-L02131				11**q**22.3
346	Reference probe 16440-L30623	18q21			
355	**PTAFR probe** 02267-L01425		1p35.3		
364	**SEMA3B probe** 03210-L02625			3p21.31	
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			3p21.31	
409	**THY1 probe** 04777-L04125				11q23.3
418	**VHL probe** 01161-L00717			3p25.3	
427	**KMT2A probe** 01637-L01175				11q23.3
436	**PARK7 probe** 02188-L01686		1p36.23		
445	**ZMYND10 probe** 03207-L02622			3p21.31	
454	**CTNNB1 probe** 00673-L00117			3p22.1	
463	**NTNG1 probe** 16354-L06009		1p13.3		
475	Reference probe 12066-L13192	20q13			
484	**CASR probe** 02683-L02148			3**q**21.1	
496 «	**CHD5 probe** 09114-L25958		1p36.31		
504	Reference probe 21229-L30802	10p11			

« 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 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.

± SNP rs55707108 could influence the probe signal at 266 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

+ An 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 1: The identity of the genes detected by the reference probes is available on request: info@mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Note 2: SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. SALSA MLPA Probemix P252-D1 NB 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		2p25.3	
136	**TP53 probe** 08304-L01158			17p13.1
142 «	**CFLAR probe** 00663-L00074		2**q**33.1	
148	Reference probe 05170-L21820	13q12		
155	Reference probe 04566-L03955	16q13		
160	**TBCD probe** 08306-L01293			17**q**25.3
168	**NF1 probe** 02514-L30629			17**q**11.2
173	**SGCA probe** 03373-L30630			17**q**21.33
178	Reference probe 04858-L04242	5p13		
184	**ERBB2 probe** 00991-L00146			17**q**12
190	**NBAS probe** 08317-L08186		2p24.3	
195	**BMPR2 probe** 12059-L09026		2**q**33.1	
202 +	Reference probe 10697-L12697	6p12		
213	**NBAS probe** 21789-L30625		2p24.3	
220	**RECQL5 probe** 04170-L03525			17**q**25.1
226	**WSB1 probe** 05736-L31080			17**q**11.1
232	**PAFAH1B1 probe** 04605-L30632			17p13.3
239	**SCN1A probe** 04543-L03932		2**q**24.3	
247	Reference probe 07695-L07419	21q22		
257	**TOP2A probe** 01055-L00628			17**q**21.2
265	**CASP8 probe** 02761-L02210		2**q**33.1	
274 «	**DDX1 probe** 08319-L08188		2p24.3	
283 ±	**RPIA probe** 05713-L05151		2p11.2	
292	Reference probe 04224-L03560	19q13		
301 «	**BMPR2 probe** 04013-L03436		2**q**33.1	
311 +	Reference probe 06425-L05951	6p22		
319 «	**DDX1 probe** 08320-L08189		2p24.3	
333	**ALK probe** 08322-L30633		2p23.2	
339	**TPO probe** 11049-L30634		2p25.3	
346	Reference probe 16440-L30623	18q21		
353 «	**MYCN probe** 12060-L09025		2p24.3	
361	Reference probe 10086-L20983	8q22		
370	**WSB1 probe** 08326-L22797			17**q**11.1
378	Reference probe 03919-L30636	15q21		
384 «	**BIRC5 probe** 03025-L02411			17**q**25.3
391	**TOB1 probe** 04778-L04126			17**q**21.33
400	**SECTM1 probe** 01088-L00647			17**q**25.3
409	**RTN4 probe** 00963-L00550		2p16.1	
420	**DYSF probe** 08839-L13359		2p13.3	
429	Reference probe 12456-L23201	22q12		
436 «	**MYCN probe** 03327-L02466		2p24.3	
445	**WSB1 probe** 08328-L09024			17**q**11.1
454	**TP53 probe** 08785-L01159			17p13.1
465	**TP53 probe** 00844-L06726			17p13.1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		Reference	Chr 2	Chr 17
475	Reference probe 12066-L13192	20q13		
486	**ALK probe** 15397-L30899		2p23.2	
493	Reference probe 14909-L27536	18p11		
500	Reference probe 21229-L29604	10p11		

« 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.

± SNP rs554374026 could influence the probe signal at 283 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

+ An 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 1: The identity of the genes detected by the reference probes is available on request: info@mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Note 2: SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 3. SALSA MLPA Probemix P253-D1 NB 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**36.3			
142	**PKP2 probe** 12061-L04788					12p11.21	
148	**SPON2 probe** 21907-L30514		4p16.3				
155	Reference probe 04566-L03955	16q13					
160	**TGFB3 probe** 21908-L31105						14**q**24.3
166 ±	**ATL1 probe** 05279-L04660						14**q**22.1
172	**PTPRD probe** 08332-L08201				9p24.1		
178	Reference probe 04858-L04242	5p13					
184	**TBX5 probe** 05687-L05129					12**q**24.21	
189 «	**IMPDH1 probe** 21909-L31106			7**q**32.1			
196	**GNRHR probe** 12062-L04183		4**q**13.2				
202 +	Reference probe 10697-L12697	6p12					
213	**TJP2 probe** 21910-L30680				9**q**21.11		
220	**COL2A1 probe** 07405-L07052					12**q**13.11	
232 ±	**WFS1 probe** 05376-L30681		4p16.1				
240	**NFKBIA probe** 13706-L31107						14**q**13.2
247	Reference probe 07695-L07419	21q22					
254 «	**KCNIP4 probe** 21878-L16046		4p15.31				
265 ⍟	**CDKN2A probe** 02238-L13510				9p21.3		
274	**CDKN2A probe** 01291-L00835				9p21.3		
283	**ERC1 probe** 06682-L06260					12p13.33	
292	Reference probe 04224-L03560	19q13					
303 ±	**EGFR probe** 05961-L20432			7p11.2			
311 +	Reference probe 06425-L05951	6p22					
322	**CDKN1B probe** 02256-L30516					12p13.1	
329	**GLRB probe** 08956-L30517		4**q**32.1				
339	**MDM2 probe** 02894-L20364					12**q**15	
346	Reference probe 16440-L30623	18q21					
352	Reference probe 11653-L22884	5q33					
360 «	**ELN probe** 12063-L22813			7**q**11.23			
368	**OCIAD1 probe** 12064-L31255		4p12				
373	Reference probe 03919-L03374	15q21					
382	**KLKB1 probe** 01136-L00694		4**q**35.2				
391 «	**MOAP1 probe** 00947-L01595						14**q**32.12
400	Reference probe 10091-L10515	8q22					
408	**KRIT1 probe** 04349-L31108			7**q**21.2			
413	**TGFBR1 probe** 04653-L31256				9**q**22.33		
421	**GHRHR probe** 07215-L13361			7p15.1			
427	**TSC1 probe** 04796-L04171				9**q**34.13		

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)					
		Reference	Chr 4	Chr 7	Chr 9	Chr 12	Chr 14
436	**POMT1 probe** 04129-L03486				9**q**34.13		
445	**IL2 probe** 00627-L00183		4**q**27				
454	**DNAI1 probe** 08059-L07840				9p13.3		
468	**PTPRD probe** 08330-L30682				9p23		
480	Reference probe 12066-L31109	20q13					
490	Reference probe 12461-L21828	22q12					
500	Reference probe 21229-L29604	10p11					

« 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, 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 (also known as SNP rs551685870) is unclear.

± SNP rs200452381, rs17290162 and rs550975729 could influence the probe signal at 166 nt, 303 nt and 232 nt, respectively. In case of apparent deletions, it is recommended to sequence the region targeted by the probe.

+ An 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 1: The identity of the genes detected by the reference probes is available on request: info@mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Note 2: SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 4. P251-C2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Chromosome 1						
166 «	04690-L07966	*GABRD*	1p36.33	CGGCGACTACGT-GGGCTCCAACCT	1,6 **M**b	01-001,946
327 «	01682-L01262	*TP73*	1p36.32	GAGACCCGGGTG-TCAGGAAAGATG	2,6 **M**b	01-003,558
496 «	09114-L25958	*CHD5*	1p36.31	GTTTCTTCTTCT-TAGGAAGGCTCA	1,8 **M**b	01-006,151
436	02188-L01686	*PARK7*	1p36.23	ATGGCGGCTATC-AGGCCCTTCCGG	2,4 **M**b	01-007,954
220	04682-L04060	*KIF1B*	1p36.22	CGTGGGGTCCTT-TTGCAGGCCCTC	18,0 **M**b	01-010,358
355	02267-L01425	*PTAFR*	1p35.3	CATCTTCATCGT-GTTCAGCTTCTT	28,5 **M**b	01-028,350
160	02876-L02343	*PLPP3*	1p32.2	CCCCTTGGACTT-TAGAACGATTTA	50,9 **M**b	01-056,817
463	16354-L06009	*NTNG1*	1p13.3	GGATAAGGCTGT-TAAGACCAGCCG	36,0 **M**b	01-107,669
130 ¤	05712-L05712	*PDE4DIP*	1**q**21.1	GCTACATCTGTT-GGAGGAGCCAAC	34,8 **M**b	01-143,658
274	07233-L06883	*LHX4*	1**q**25.2	CATGGCCCCGCA-TGGTCCCCTCTC	46,0 **M**b	01-178,502
320	12058-L03618	*LIN9*	1**q**42.12	GGCCTTCTCGAT-TTTTTATGACCC	17,4 **M**b	01-224,521
382	21295-L30115	*AKT3*	1**q**44	TTGCCTCTGCAG-TCTGTCTGCTAC	-	01-241,876
Chromosome 3						
418 #	01161-L00717	*VHL*	3p25.3	CTAGTCAAGCCT-GAGAATTACAGG	20,5 **M**b	03-010,166
196	03861-L03610	*TGFBR2*	3p24.1	CTGTGACAACCA-GAAATCCTGCAT	10,6 **M**b	03-030,661
454	00673-L00117	*CTNNB1*	3p22.1	GGCCATGGAACC-AGACAGAAAAGC	9,0 **M**b	03-041,241
364	03210-L02625	*SEMA3B*	3p21.31	ACCTGGACAACA-TCAGCAAGCGGG	60,4 kb	03-050,283
404	03991-L30512	*RASSF1*	3p21.31	TCCTGCAGAAGT-ACTCCTATTGCC	12,8 kb	03-050,343
445	03207-L02622	*ZMYND10*	3p21.31	AAGACACTGTCT-TGGACTTGGTAG	26,9 **M**b	03-050,356
283	06447-L05973	*ROBO2*	3p12.3	GGAAGCTACGTT-TGTGTTGCGAGG	46,3 **M**b	03-077,230
484	02683-L02148	*CASR*	3**q**21.1	GCCCAGATGACT-TCTGGTCCAATG	25,1 **M**b	03-123,485
396	08544-L30513	*ZIC1*	3**q**24	ATGCACTCTATG-TGTTCAGGAAGC	31,8 **M**b	03-148,616
211	03826-L03222	*PIK3CA*	3**q**26.32	ACACGTTCATGT-GCTGGATACTGT	-	03-180,430
Chromosome 11						
232	16712-L19296	*LMO1*	11p15.4	GCCACATTAGAA-CTTCTCCGTCCT	39,0 kb	11-008,203
226 «	16709-L19293	*LMO1*	11p15.4	TTCACTCCTGAA-TGTAATTCTAGC	547,8 kb	11-008,242
142 «	06679-L06257	*DENND2B*	11p15.4	GCCACCACTAGT-ACCATGAGTCCC	8,6 **M**b	11-008,789
254 «	21876-L30842	*ABCC8*	11p15.1	CACTTCCAGATT-TAACCTGGACCC	17,7 **M**b	11-017,373
266 ±	02245-L30511	*CD44*	11p13	CCCGCGCCCTCC-GTTCGCTCCGGA	13,0 **M**b	11-035,117
190	05918-L05363	*PTPRJ*	11p11.2	GGGGAGACAGAT-TCTTCCAATCTC	19,0 **M**b	11-048,106
172	06819-L07011	*GSTP1*	11**q**13.2	ACCAGTCCAATA-CCATCCTGCGTC	32,6 **M**b	11-067,109

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
301	08313-L08182	*CNTN5*	11**q**22.1	ATTCTTGTTGCA-TGGAAACACATT	4,7 **M**b	11-099,684
184 #	00559-L00128	*CASP1*	11**q**22.3	CCGCACACGTCT-TGCTCTCATTAT	3,3 **M**b	11-104,406
337	02664-L02131	*ATM*	11**q**22.3	TTTTTCCGATGC-TGTTTGGATAAA	7,2 **M**b	11-107,684
238	01640-L01178	*CADM1*	11**q**23.2	GATCCGGGGAAA-GCAAAACCCGAA	3,0 **M**b	11-114,881
427	01637-L01175	*KMT2A*	11**q**23.3	GGACCCCGGATT-AAACATGTCTGC	612,8 kb	11-117,853
149	01662-L30621	*HMBS*	11**q**23.3	CATCTCTATAGA-GTGGACCTGGTT	329,5 kb	11-118,466
409	04777-L04125	*THY1*	11**q**23.3	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 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.

± SNP rs55707108 could influence the probe signal at 266 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

[a] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 5. P252-D1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Chromosome 2						
131	06296-L25684	*TMEM18*	2p25.3	TCCTCACCTGCT-TGTCCTCCCGAA	773,4 kb	02-000,666
339	11049-L30634	*TPO*	2p25.3	GATGACCGCTAT-TCTGACCTCCTG	13,8 **M**b	02-001,439
190	08317-L08186	*NBAS*	2p24.3	GTCCCTCCTGCT-TCCATCTCTGAA	238,6 kb	02-015,237
213	21789-L30625	*NBAS*	2p24.3	CTGGTTCTCTGT-GACAATTTGGTT	185,0 kb	02-015,475
274 «	08319-L08188	*DDX1*	2p24.3	TCAAAGCAGAGA-AGTAAAGGAATG	14,7 kb	02-015,660
319 «	08320-L08189	*DDX1*	2p24.3	GTGTCAACTGGA-AAGCTGAACTTA	328,3 kb	02-015,675
353 «	12060-L09025	*MYCN*	2p24.3	CTGTCACCACAT-TCACCATCACTG	0,2 kb	02-016,003
436 «	03327-L02466	*MYCN*	2p24.3	TGCACCCCCACA-GAAGAAGATAAA	13,3 **M**b	02-016,003
486	15397-L30899	*ALK*	2p23.2	TTTCTCTTGGAT-ATATGCCATACC	520,0 kb	02-029,274
333	08322-L30633	*ALK*	2p23.2	ATCTCACCTGGA-TAATGAAAGACT	25,3 **M**b	02-029,794
409	00963-L00550	*RTN4*	2p16.1	CTGGAGAGACAT-TAAGAAGACTGG	16,7 **M**b	02-055,068
420	08839-L13359	*DYSF*	2p13.3	TGCCATGAAGCT-GGTGAAGCCCTT	17,0 **M**b	02-071,767
283 ± #	05713-L05151	*RPIA*	2p11.2	TGGTTCTACAAT-TGTCCATGCTGT	77,8 **M**b	02-088,779
239	04543-L03932	*SCN1A*	2**q**24.3	ATAGGCCACATT-CAAAGGATGGAT	35,1 **M**b	02-166,564
142 «	00663-L00074	*CFLAR*	2**q**33.1	TGTCTGTCGGGG-ACTTGGCTGAAC	127,9 kb	02-201,703
265	02761-L02210	*CASP8*	2**q**33.1	TGTCCAGCGCTC-GGGCTTTAGTTT	1,1 **M**b	02-201,831
195	12059-L09026	*BMPR2*	2**q**33.1	GGATTTGTTGTT-TTCGAAATCAGA	165,5 kb	02-202,950
301 «	04013-L03436	*BMPR2*	2**q**33.1	TTGAGGATATGC-AGGTTCTCGTGT	-	02-203,115
Chromosome 17						
232 #	04605-L30632	*PAFAH1B1*	17p13.3	CTGTTCTGCAGA-TATGACCATTAA	5,0 **M**b	17-002,520
454	08785-L01159	*TP53*	17p13.1	TTCCGAGAGCTG-AATGAGGCCTTG	3,1 kb	17-007,515
136	08304-L01158	*TP53*	17p13.1	CTGTCCTGGGAG-AGACCGGCGCAC	2,5 kb	17-007,518
465	00844-L06726	*TP53*	17p13.1	CATCTACAGTCC-CCCTTGCCGTCC	15,1 **M**b	17-007,520
370	08326-L22797	*WSB1*	17**q**11.1	CTCTTCTCTGTT-GTTGGGTCCGCA	17,2 kb	17-022,645
226	05736-L31080	*WSB1*	17**q**11.1	ATTGATGAGGAT-TATCCAGTGCAA	0,8 kb	17-022,663
445	08328-L09024	*WSB1*	17**q**11.1	GTCGCATGTCAA-TCCGAAGAGTGA	3,9 **M**b	17-022,663
168 #	02514-L30629	*NF1*	17**q**11.2	TCTTTCCTTCAT-AAGTGACGGCAA	8,5 **M**b	17-026,612
184	00991-L00146	*ERBB2*	17**q**12	GGTGCAGGGCTA-CGTGCTCATCGC	704,6 kb	17-035,118
257	01055-L00628	*TOP2A*	17**q**21.2	AAGCCCTTCAAT-GGAGAAGATTAT	9,8 **M**b	17-035,823

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
173	03373-L30630	*SGCA*	17**q**21.33	CCATGTTCAATG-TGCACACAGGTG	688,5 kb	17-045,608
391	04778-L04126	*TOB1*	17**q**21.33	TGTCAACATTTT-TGGTGAAGAACT	24,8 **M**b	17-046,296
220	04170-L03525	*RECQL5*	17**q**25.1	GGCTGCAAATGT-TGTGGTCAAGTG	2,6 **M**b	17-071,136
384 «	03025-L02411	*BIRC5*	17**q**25.3	GCATTCGTCCGG-TTGCGCTTTCCT	4,1 **M**b	17-073,724
400	01088-L00647	*SECTM1*	17**q**25.3	TCTTCATCCTCT-TGGTCGCTCTGG	577,8 kb	17-077,874
160	08306-L01293	*TBCD*	17**q**25.3	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.

± SNP rs554374026 could influence the probe signal at 283 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

[a] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 6. P253-D1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Chromosome 4						
148	21907-L30514	*SPON2*	4p16.3	CTTCCCCAAGCA-GTACCCCCTGTT	5,2 **M**b	04-001,156
232 ±	05376-L30681	*WFS1*	4p16.1	CTCAATGCCACA-GCCTCGTTGGAG	15,2 **M**b	04-006,330
254 «	21878-L16046	*KCNIP4*	4p15.31	GTGGAAAGCATT-TCGGCTCAGCTG	27,0 **M**b	04-021,559
368	12064-L31255	*OCIAD1*	4p12	ATGCTTCCTCAT-TATGAGCCAATT	19,8 **M**b	04-048,549
196	12062-L04183	*GNRHR*	4**q**13.2	TGGAACATTACA-GTCCAATGGTAT	55,3 **M**b	04-068,302
445	00627-L00183	*IL2*	4**q**27	ACAATGTACAGG-ATGCAACTCCTG	34,7 **M**b	04-123,597
329	08956-L30517	*GLRB*	4**q**32.1	TATTGCTTGCCT-TCTCTTTGGGTT	29,1 **M**b	04-158,293
382	01136-L00694	*KLKB1*	4**q**35.2	ATGCCCAATACT-GCCAGATGAGGT	-	04-187,390
Chromosome 7						
421	07215-L13361	*GHRHR*	7p15.1	TTCCTCAACCAA-GAGGTGTGTGAT	24,2 **M**b	07-030,983
303 ±	05961-L20432	*EGFR*	7p11.2	TCATGGGAGAAA-ACAACACCCTGG	17,9 **M**b	07-055,201
360 «	12063-L22813	*ELN*	7**q**11.23	ACCTCATCAACG-TTGGTGCTACTG	18,6 **M**b	07-073,121
408	04349-L31108	*KRIT1*	7**q**21.2	CAATCCAAACCT-TTTAAATGGACA	36,1 **M**b	07-091,694
189 « #	21909-L31106	*IMPDH1*	7**q**32.1	GGGGCCTCCGTA-GTGGCGGCCAGC	27,5 **M**b	07-127,822
136	06358-L05874	*SHH*	7**q**36.3	CAAGGCACATAT-CCACTGCTCGGT	-	07-155,292
Chromosome 9						
172	08332-L08201	*PTPRD*	9p24.1	CACAAGGGAGCA-TCATACGTCTTC	1,5 **M**b	09-008,476
468	08330-L30682	*PTPRD*	9p23	TAGAGGTGTCTG-ACTGACAGCATG	12,0 **M**b	09-009,929
274	01291-L00835	*CDKN2A*	9p21.3	TGAAAGAACCAG-AGAGGCTCTGAG	27,3 kb	09-021,958
265 ◎	02238-L13510	*CDKN2A*	9p21.3	AGACCGGAGAGA-GAACGTACGCCG	12,5 **M**b	09-021,985
454	08059-L07840	*DNAI1*	9p13.3	ACTGAAGTGGAA-GAGAGTCCAGAT	36,6 **M**b	09-034,449
213	21910-L30680	*TJP2*	9**q**21.11	CGTTTTTTATAA-GAAGCCACTTTG	29,9 **M**b	09-071,041
413	04653-L31256	*TGFBR1*	9**q**22.33	GATGGGTCAGAA-GGTACAAGATCA	32,4 **M**b	09-100,950
436	04129-L03486	*POMT1*	9**q**34.13	GGAGCTCCACTT-TTCTCATTGTGC	1,4 **M**b	09-133,373
427	04796-L04171	*TSC1*	9**q**34.13	ACCCAGCAAGTC-TGTCGACTGGAC	-	09-134,770
Chromosome 12						
283	06682-L06260	*ERC1*	12p13.33	GAACGGGACAAT-GCAGAACTGCAG	11,3 **M**b	12-001,470
322	02256-L30516	*CDKN1B*	12p13.1	GACTCCGACGCC-GGCAAGGTTTGG	20,1 **M**b	12-012,762
142 #	12061-L04788	*PKP2*	12p11.21	GAAGATGTGACG-GACTCATTGACT	13,8 **M**b	12-032,868
220	07405-L07052	*COL2A1*	12**q**13.11	CACAGGGTCCTT-CTGGAGACCAAG	20,8 **M**b	12-046,657
339	02894-L20364	*MDM2*	12**q**15	CGAGATCCTGCT-GCTTTCGCAGCC	45,8 **M**b	12-067,488
184	05687-L05129	*TBX5*	12**q**24.21	GCCTGACGCAAA-AGACCTGCCCTG	-	12-113,326

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Chromosome 14						
240	13706-L31107	*NFKBIA*	14**q**13.2	CTACCAGGGCTA-TTCTCCCTACCA	15,2 **M**b	14-034,941
166 ±	05279-L04660	*ATL1*	14**q**22.1	AGCCAGTGAAAA-AGGCAGGACCAG	25,4 **M**b	14-050,124
160	21908-L31105	*TGFB3*	14**q**24.3	TGCACCCAGGAA-AACACCGAGTCG	17,2 **M**b	14-075,517
391 «	00947-L01595	*MOAP1*	14**q**32.12	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, 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 (also known as SNP rs551685870) is unclear.

± SNP rs200452381, rs17290162 and rs550975729 could influence the probe signal at 166 nt, 303 nt and 232 nt, respectively. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

[a] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

- **P037 CLL-1**: Contains four probes for *MYCN* and one additional probe for *ALK*.
- **P056 TP53**: Contains at least one probe for each exon of *TP53*.
- **P088 Oligodendroglioma 1p-19q**: Contains probes for the 1p and 19q chromosomal arms, *CDKN2A* & *CDKN2B* genes and *IDH1* p.R132H/C and *IDH2* p.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 CDKN2A/2B 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 specific 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 specific 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 specific 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/D1-04 – 31 January 2023 (04P)
- Corrections made in the positive sample table on page 4: NA10985 has a heterozygous deletion of *VHL*, instead of a duplication and one typo corrected.

Version C2/D1/D1-03 – 30 June 2022 (04P)
- Product description rewritten and adapted to a new template.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- New warnings for SNPs influencing probe signals added and salt warnings adjusted based on newest data in Tables 1 to 6.
- Gene name of *ST5* updated to *DENND2B*.
- Information about positive control DNA samples added.
- New references added.
- Related probemixes section updated.

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.

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