Product Description

SALSA® MLPA® Probemix P301-B1 Medulloblastoma mix 1

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 9.

This SALSA MLPA probemix is for basic research and intended for experienced MLPA users only! This probemix enables you to quantify genes or chromosomal regions in which the occurrence of copy number changes is not yet well-established and the relationship between genotype and phenotype is not yet clear. Since it will not provide you with clear cut answers, interpretation of results can be complicated. MRC Holland recommends thoroughly screening any available literature. Suggestions from specialists for improvement of this product or product description are highly appreciated.

Catalogue numbers:

- **P301-025R:** SALSA MLPA Probemix P301 Medulloblastoma mix 1, 25 reactions.
- **P301-050R:** SALSA MLPA Probemix P301 Medulloblastoma mix 1, 50 reactions.
- **P301-100R:** SALSA MLPA Probemix P301 Medulloblastoma mix 1, 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 Probemix P301 Medulloblastoma mix 1 is a **research use only (RUO)** assay for the detection of deletions or gains in chromosomes 6, 14q, 16 and 17, which are suggested to be associated with medulloblastoma.

Medulloblastoma (MB) is the most common paediatric primary central nervous system (CNS) cancer type, accounting for 15% to 20% of CNS tumours in patients under the age of 20. It is a highly invasive embryonal neuroepithelial tumour that arises in the cerebellum and has a tendency to disseminate throughout the CNS early in its course. Overall survival is 50-60% at five years, although this decreases to 30% in the longer term due to local recurrence and/or metastasis. There are four distinct molecular subtypes of MB (WNT, sonic hedgehog (SHH), Group 3, and Group 4), which can be used for patient risk stratification and that have the potential to guide new therapeutic strategies for the treatment of MB (Taylor et al. 2012). These molecular subtypes of MB include characteristic and recurrent copy number alterations, which are covered by the P301, P302 and P303 Medulloblastoma probemixes.

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 SALSA MLPA Probemix P301-B1 Medulloblastoma mix 1 contains 47 MLPA probes with amplification products between 126 and 492 nucleotides (nt). This includes 35 probes for the chromosomes 6, 14q, 16 and 17. In addition, 12 reference probes are included that target relatively copy number stable regions in various

cancer types including medulloblastoma. Partial probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and online (www.mrcholland.com).

This 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 cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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. Samples from the Coriell Institute have been tested with this P301-B1 probemix at MRC Holland and can be used as positive control samples to detect various copy number alterations (CNAs) as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Chromosomal position of CNA*	Altered target genes in P301-B1	Expected CNA
Germline samples from Coriell Institute.			
NA12721	6p22.3	*E2F3, SOX4*	Heterozygous duplication
NA01221	6q15	*MAP3K7*	Heterozygous duplication
NA09367	6q23.2-q23.3	*SGK1, MYB*	Heterozygous duplication
NA07994	6q23.3-q26	*MYB, MYCT1, MAP3K4*	Heterozygous duplication
NA06802	6q26	*MAP3K4*	Heterozygous deletion
NA05966	14q23.1-q24.3	*OTX2, MLH3*	Heterozygous duplication
NA02325	16p13.3	*AXIN1, MEFV*	Heterozygous duplication
NA09687	16p13.3	*AXIN1*	Heterozygous deletion
	16q22.3-q24.3	*ZFHX3, FANCA*	Heterozygous duplication
NA13284	16p13.3	*AXIN1*	Heterozygous duplication
NA08039	16p13.3	*MEFV*	Heterozygous duplication
NA05875	16p11.2	*TGFB1I1*	Heterozygous deletion
NA12074	16q22.3	*ZFHX3*	Heterozygous deletion
NA06047	17p13.2-p13.3	*HIC1, PAFAH1B1, ATP2A3*	Heterozygous deletion
NA13476	17p11.2	*PRPSAP2*	Heterozygous deletion
NA16445	17q25.3	*TK1, BIRC5, ARHGDIA, RAC3*	Heterozygous duplication
Cancer cell line sample from Leibniz Institute DSMZ.			
HD-MB03 (ACC-740)	6q23.2-q26	*SGK1, MYB, MYCT1, MAP3K4*	Heterozygous deletion
	17p11.2-p13.3	*HIC1, PAFAH1B1, ATP2A3, TP53, RCVRN, PRPSAP2*	
	14q23.1	*OTX2*⁺	Gain
	17q12-q25.3	*ERBB2, RARA, TOP2A, RPS6KB1, PPM1D, AXIN2, TK1, BIRC5, ARHGDIA, RAC3*	

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by this P301-B1 Medulloblastoma mix 1 probemix.
⁺ High signal, indicating amplification, was observed for 10204-L13655 probe targeting *OTX2* gene.

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. 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 these criteria are fulfilled, the following cut-off values for the 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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 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 or in or near the *RAC3, HIC1, AXIN1* and *CDK5R1* genes. 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 or flanking 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 gain or amplification of one or more genes 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.

P301 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 cancer types, the most common genetic aberrations are small (point) mutations, which will not be detected by using SALSA MLPA Probemix P301 Medulloblastoma mix 1.
- 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, 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 P301-B1 Medulloblastoma mix 1

Length (nt)	SALSA MLPA probe	Reference	Chromosomal position (hg18)				Location (hg18) in kb
			chr. 6	chr. 14q	chr. 16	chr. 17	
64-105	Control fragments – see table in probemix content section for more information						
126 ¥	Reference probe 18709-L21698	5q31					05-132,038
131 «	**RAC3 probe** 11724-L14159					17**q**25.3	17-077,585
142	Reference probe 08143-L08022	5p12					05-044,337
148	**ERBB2 probe** 00675-L00572					17**q**12	17-035,118
154	**ADGRG1 probe** 10195-L10655				16**q**13		16-056,247
166	**MEFV probe** 05451-L04888				16p13.3		16-003,237
173 #	**PAFAH1B1 probe** 04175-L03579					17p13.3	17-002,520
178 ¥	**RARA probe** 10209-L32138					17**q**21.2	17-035,762
184 *	Reference probe 04857-L32200	5p13					05-037,080
190	**OTX2 probe** 10204-L13655			14**q**23.1			14-056,339
196	**TK1 probe** 10217-L10687					17**q**25.3	17-073,682
202	**TGFB1I1 probe** 13121-L10718				16p11.2		16-031,393
208 ¥	**NLK probe** 21533-L32170					17**q**11.2	17-023,520
212 «	**HIC1 probe** 13122-L00949					17p13.3	17-001,905
220	**MLH3 probe** 02107-L14737			14**q**24.3			14-074,555
226	**MYCT1 probe** 10201-L10663		6**q**25.2				06-153,085
232 ¥ «	**AXIN1 probe** 21510-L10649				16p13.3		16-000,278
243 *	Reference probe 19134-L25333	21q22					21-037,920
250	**ARHGDIA probe** 08097-L12510					17**q**25.3	17-077,420
257	**TOP2A probe** 01055-L00628					17**q**21.2	17-035,823
265	**SOX4 probe** 10214-L10681		6p22.3				06-021,702
274	**ATP2A3 probe** 10186-L10646					17p13.2	17-003,780
283	**RPS6KB1 probe** 08617-L08629					17**q**23.1	17-055,325
293	**MYB probe** 10245-L00359		6**q**23.3				06-135,555
301 ‡	**TP53 probe** 02379-L13860					17p13.1	17-007,518
310	**AXIN2 probe** 00440-L14744					17**q**24.1	17-060,956
317 *	Reference probe 06580-L30649	2q24					02-165,907
325 ¥	**BIRC5** probe 03717-L32161					17**q**25.3	17-073,722
332 ¥	**PRPSAP2** probe 01454-L14433					17p11.2	17-018,710
337	**PPM1D** probe 03195-L02652					17**q**23.2	17-056,056
346	**SGK1** probe 10212-L10679		6**q**23.2				06-134,537
355	Reference probe 06711-L06315	15q24					15-070,455
364	**MAP3K4** probe 10196-L14739		6**q**26				06-161,375
373	**MMP2** probe 04765-L04113				16**q**12.2		16-054,074
381	**VEGFA** probe 12579-L11003		6p21.1				06-043,846
391 ¥ «	**CDK5R1** probe 22788-L10650					17**q**11.2	17-027,840
400	**FANCA** probe 04181-L14740				16**q**24.3		16-088,333
409 *	Reference probe 09720-L32156	12q24					12-116,181
418	**MAP3K7** probe 10198-L10658		6**q**15				06-091,327
424	**RCVRN probe** 10211-L10677					17p13.1	17-009,745
432 ¥	Reference probe 21891-L12107	4q12					04-052,590
445	**ZFHX3 probe** 04738-L04086				16**q**22.3		16-071,551
454	Reference probe 07607-L07292	15q26					15-097,300
465 *	Reference probe 12460-L32162	22q12					22-032,110
472	**E2F3 probe** 12029-L12891		6p22.3				06-020,588
481 *	Reference probe 08614-L32159	12p12					12-014,820
492 *	Reference probe 17001-L18577	20q11					20-034,954

* New in version B1.
¥ Changed in version B1. Minor alteration, 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.

‡ This probe may be non-specific. Apparent deletions/gains of this TP53 probe always need to be confirmed with another probemix (e.g. P056 TP53) or with another technique.
# 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P301-B1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
Chromosome 6					
472	12029-L12891	***E2F3***	6p22.3	CTTTGCCTGTAA-TAAGAAAGTGCG	1.1 Mb
265	10214-L10681	***SOX4***	6p22.3	CCAGCATTCGAG-AAACTCCTCTCT	22.1 Mb
381	12579-L11003	***VEGFA***	6p21.1	GTGGATTTTGGA-AACCAGCAGAAA	47.5 Mb
418	10198-L10658	***MAP3K7***	6**q**15	GTGTTTACAGTG-TTCCCAAGGAGT	43.2 Mb
346	10212-L10679	***SGK1***	6**q**23.2	GAGGATGGGTCT-GAACGACTTTAT	1.0 Mb
293	10245-L00359	***MYB***	6**q**23.3	ATGCGTCGGAAG-GTCGAACAGGAA	17.5 Mb
226	10201-L10663	***MYCT1***	6**q**25.2	CACTTCCCACAG-TCTGAGCCGTCC	8.3 Mb
364	10196-L14739	***MAP3K4***	6**q**26	AAACGCATGTCA-ACCAAACATCAG	-
Chromosome 14q					
190	10204-L13655	***OTX2***	14**q**23.1	TCCTGCATGCAG-AGGTCCTATCCC	18.2 Mb
220	02107-L14737	***MLH3***	14**q**24.3	TGCCGCCTTATT-GAAGCTCTGTCC	-
Chromosome 16					
232 «	21510-L10649	***AXIN1***	16p13.3	GATCATCGGCAA-AGTGGAGAAGGT	3.0 Mb
166	05451-L04888	***MEFV***	16p13.3	GGCCTCACTGGA-GGACGTGGGCCA	28.2 Mb
202	13121-L10718	***TGFB1I1***	16p11.2	CAGGAACTTAAT-GCCACTCAGTTC	22.7 Mb
373	04765-L04113	***MMP2***	16**q**12.2	TGCAACCTGTTT-GTGCTGAAGGAC	2.2 Mb
154	10195-L10655	***ADGRG1***	16**q**13	GATTGTGGTACA-GAACACCAAAGT	15.3 Mb
445	04738-L04086	***ZFHX3***	16**q**22.3	ATTCTTAGCAAT-AAGAACATCTCC	16.8 Mb
400	04181-L14740	***FANCA***	16**q**24.3	CATGTTGCTGTG-GACATGTACTTG	-
Chromosome 17					
212 «	13122-L00949	***HIC1***	17p13.3	AGAGTGTGCGGA-AAGCGCGGCGGG	0.6 Mb
173 #	04175-L03579	***PAFAH1B1***	17p13.3	CTGTTCTGCAGA-TATGACCATTAA	1.3 Mb
274	10186-L10646	***ATP2A3***	17p13.2	GAGGTGTTCGAG-TCACGCTTCCCC	3.7 Mb
301 ‡	02379-L13860	***TP53***	17p13.1	ACTGCCCAACAA-CACCAGCTCCTC	2.2 Mb
424	10211-L10677	***RCVRN***	17p13.1	CTTCCAGACGAT-GAAAACACGCCG	9.0 Mb
332	01454-L14433	***PRPSAP2***	17p11.2	TAGAAACCAAGA-TGAACATAACCA	4.8 Mb
208	21533-L32170	***NLK***	17**q**11.2	AGAATTAGATGA-ATCCCGTCATAT	4.3 Mb
391 «	22788-L10650	***CDK5R1***	17**q**11.2	TCATGAGCTCAA-AGATGCTGCAGA	7.3 Mb
148	00675-L00572	***ERBB2***	17**q**12	GGTGCAGGGCTA-CGTGCTCATCGC	0.6 Mb
178	10209-L32138	***RARA***	17**q**21.2	TCTCTGGACATT-GACCTCTGGGAC	60.2 **k**b
257	01055-L00628	***TOP2A***	17**q**21.2	AAGCCCTTCAAT-GGAGAAGATTAT	19.5 Mb
283	08617-L08629	***RPS6KB1***	17**q**23.1	CAGGAGTGTTTG-ACATAGACCTGG	0.7 Mb
337	03195-L02652	***PPM1D***	17**q**23.2	TGTGGTCATCAT-TCGGGGCATGAA	4.9 Mb
310	00440-L14744	***AXIN2***	17**q**24.1	CCCGAAGCTCTT-GTGAACTGTCTT	12.7 Mb
196	10217-L10687	***TK1***	17**q**25.3	ATCTTTCACCAA-GATGGGTGGCAC	40.2 **k**b
325	03717-L32161	***BIRC5***	17**q**25.3	CTCTACATTCAA-GAACTGGCCCTT	3.7 Mb
250	08097-L12510	***ARHGDIA***	17**q**25.3	CAGGTTAACCGA-GAGATAGTGTCC	0.2 Mb
131 «	11724-L14159	***RAC3***	17**q**25.3	GGCAGGATCCTG-TCCTCTCTGCCG	-

« 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 may be non-specific. Apparent deletions/gains of this TP53 probe always need to be confirmed with another probemix (e.g. P056 TP53) or with another technique.

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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 3. P301-B1 reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
317	06580-L30649	*SCN2A*	2q24	AACTTGGTTTGG-CAAATGTGGAAG	-
432	21891-L12107	*SGCB*	4q12	TGTAGAAAACAA-CAAAACTTCTAT	-
184	04857-L32200	*NIPBL*	5p13	CTGCAATGTTGC-AAAAATCCTAGA	7.3 Mb
142	08143-L08022	*FGF10*	5p12	GATGCTGCCAAT-TCAAGGTTTGTG	87.7 Mb
126	18709-L21698	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	-
481	08614-L32159	*H2AFJ*	12p12	ACTCAGGACCAA-GTTCTGGGAAGA	101.4 Mb
409	09720-L32156	*NOS1*	12q24	AGAATATGACAT-TGTGCACCTGGA	-
355	06711-L06315	*HEXA*	15q24	AGGCACTCCACT-TCCTCCTCGAGC	26.8 Mb
454	07607-L07292	*IGF1R*	15q26	CATGGTAGCCGA-AGATTTCACAGT	-
492	17001-L18577	*SAMHD1*	20q11	CCCTGTCACCTC-AAGTTTGAGGAT	-
243	19134-L25333	*KCNJ6*	21q22	CTCGAAGCTCCT-ACATCACCAGTG	-
465	12460-L32162	*LARGE1*	22q12	AGGAATAGCTGC-ACCTTCGAACCT	-

Related SALSA MLPA probemixes

P302 Medulloblastoma mix 2	Contain probes for chromosomes 2, 3, 7 and 9.
P303 Medulloblastoma mix 3	Contain probes for chromosomes 1, 4q, 5q, 8, 10 and 20.
P251-P252-P253 Neuroblastoma	Contain probes for multiple chromosomal regions that frequently show CNAs in neuroblastoma tumours (chromosomes 1, 2, 3, 4, 7, 9, 11, 12, 14 and 17).

References

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Selected publications using SALSA MLPA Probemix P301 Medulloblastoma mix 1

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P301 product history	
Version	*Modification*
B1	Several reference probes have been replaced and several probes have a change in length but no change in the sequence detected.
A2	Control fragments (QDX) have been optimized, one reference probe has been replaced and one added.
A1	First release.

Implemented changes in the product description

Version B1-03 – 23 September 2025 (04P)
- Adjusted remarks regarding availability of complete probe sequences.
- Adjusted implemented changes for Version B1-02 regarding 'Positive control DNA samples' section: added "and removed one Coriell cell line sample".

Version B1-02 – 08 April 2024 (04P)
- Product description rewritten and adapted to a new template.
- Added one cancer cell line sample in 'Positive control DNA samples' section and removed one Coriell cell line sample.
- Added P301 specific note on page 4.
- "Location (hg18) in kb" column added in Table 1.
- Removed the "« Probe located in or near a GC-rich region…" remark in Table 1 and 2 for SOX4 (10214-L10681), MYB (10245-L00359) and ARHGDIA (08097-L12510) probes.
- Renamed Tabel 2a and 2b to Table 2 and 3, respectively.
- Added new references in section 'Selected publications using SALSA MLPA Probemix P301 Medulloblastoma mix 1'.

Version B1-01 — 16 September 2020 (02P)
- Joint product description for P301, P302 and P303 probemixes is now divided into separate product descriptions.
- Product description adapted to a new product version and to a new template (version number changed, changes in Table 1 and Table 2).
- Various minor textual or layout changes.
- Name of *GPR56* gene is updated in Tables 1 & 2a to *ADGRG1* according to the new HUGO nomenclature.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

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