Product Description SALSA® MLPA® Probemix P302-A3 Medulloblastoma mix 2

To be used with the MLPA General Protocol.

Version A3. As compared to version A2, four reference probes are replaced, and several probes have a change in length but no change in the sequence targeted. For complete product history see page 7.

Catalogue numbers:
- **P302-025R:** SALSA MLPA Probemix P302 Medulloblastoma mix 2, 25 reactions.
- **P302-050R:** SALSA MLPA Probemix P302 Medulloblastoma mix 2, 50 reactions.
- **P302-100R:** SALSA MLPA Probemix P302 Medulloblastoma mix 2, 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.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.

This SALSA MLPA probemix is for basic research and intended for experienced MLPA users only! This probemix enables the quantification of genes or chromosomal regions in which the occurrence or relevance of copy number changes is not yet well-established. Interpretation of results may be complicated, and MRC Holland may only be able to provide basic support.

General information: The SALSA MLPA Probemix P302 Medulloblastoma mix 2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the chromosomes 2, 3, 7 and 9, which are thought to be associated with medulloblastoma.

Medulloblastoma (MB) is the most common paediatric primary central nervous system (CNS) tumour and accounts for between 15% and 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 identify new therapeutic strategies for the treatment of MB (Taylor et al. 2012). These molecular subtypes of MB include also 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 P302-A3 Medulloblastoma mix 2 contains 50 MLPA probes with amplification products between 121 and 500 nucleotides (nt). This includes 37 probes for the chromosomes 2, 3, 7 and 9. In addition, 13 reference probes are included that target relatively copy number stable regions in various cancer types including medullobalstoma. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 2b and online (www.mlpa.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.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 nt and 96 nt fragment 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.mlpa.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 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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. Sample ID numbers NA10401, NA01353, NA04409, NA00501, NA00945, NA09216, NA01229, NA22770, NA04127, NA10985, NA11428, NA08778, NA03563, NA22976, NA07081, NA08763, NA12590, NA10160, NA12519, NA07412, NA01220, NA10313, NA02819, NA01750, NA03226, NA05067 and NA13685 from the Coriell Institute have been tested with this P302-A3 probemix at MRC-Holland and can be used as a positive control samples to detect deletions and duplications in multiple genomic regions as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P302-A3	Expected CNA
NA10401	Coriell Institute	2p25.3-q37.3	*TMEM18, NBAS, MYCN, ALK, RTN4, RPIA, IL1RN, RPRM, BMPR2, ATG4B*	Heterozygous duplication
NA01353	Coriell Institute	2p23.2-p25.3	*TMEM18, NBAS, MYCN, ALK*	Heterozygous duplication
NA04409	Coriell Institute	2p24.3-p25.3	*TMEM18, NBAS, MYCN*	Heterozygous duplication
NA00501	Coriell Institute	2p25.3	*TMEM18*	Heterozygous deletion
NA00945	Coriell Institute	2p24.3	*NBAS, MYCN*	Heterozygous deletion
NA09216	Coriell Institute	2p24.3	*NBAS, MYCN*	Heterozygous deletion
NA01229	Coriell Institute	2q33.1-q37.3	*BMPR2, ATG4B*	Heterozygous duplication
NA22770	Coriell Institute	2q37.3	*ATG4B*	Heterozygous deletion
NA04127	Coriell Institute	3p21.31-p26.3	*CRBN, PPARG, CTNNB1, RASSF1*	Heterozygous duplication
NA10985	Coriell Institute	3p26.3	*CRBN*	Heterozygous deletion

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P302-A3	Expected CNA
NA11428	Coriell Institute	3p26.3	*CRBN*	Heterozygous deletion
		3q24-q27.1	*ZIC1, SLITRK3, MCCC1*	Heterozygous duplication
NA08778	Coriell Institute	3q13.33	*CASR*	Heterozygous deletion
NA03563	Coriell Institute	3q13.33-q27.1	*CASR, ZIC1, SLITRK3, MCCC1*	Heterozygous duplication
NA22976	Coriell Institute	3q27.1	*MCCC1*	Heterozygous duplication
		9p24.1-q34.3	*PTPRD, CDKN2A, CDKN2B, IGFBPL1, TRPM3, ALDOB, DEC1, EHMT1*	Heterozygous duplication
NA07081	Coriell Institute	7p11.2-p22.3	*MAFK, GHRHR, EGFR*	Heterozygous duplication
NA08763	Coriell Institute	7p15.1	*GHRHR*	Heterozygous deletion
NA12590	Coriell Institute	7q11.23	*ELN*	Heterozygous deletion
NA10160	Coriell Institute	7q11.23-q21.2	*ELN, CDK6*	Heterozygous deletion
NA12519	Coriell Institute	7q32.1	*IMPDH1*	Heterozygous triplication / homozygous duplication
NA07412	Coriell Institute	7q36.3	*SHH*	Heterozygous deletion
NA01220	Coriell Institute	7q36.3	*SHH*	Heterozygous duplication
NA10313	Coriell Institute	7q36.3	*SHH*	Heterozygous deletion
NA02819	Coriell Institute	9p21.3-p24.1	*PTPRD, CDKN2A, CDKN2B*	Heterozygous duplication
NA01750	Coriell Institute	9p21.3-p24.1	*PTPRD, CDKN2A, CDKN2B*	Heterozygous duplication
NA03226	Coriell Institute	9p13.1-p24.1	*PTPRD, CDKN2A, CDKN2B, IGFBPL1*	Heterozygous duplication
NA05067	Coriell Institute	9p13.1-p24.1	*PTPRD, CDKN2A, CDKN2B, IGFBPL1*	Heterozygous duplication
NA13685	Coriell Institute	9q34.3	*EHMT1*	Heterozygous duplication

* 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 this P302-A3 Medulloblastoma mix 2 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.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 each individual probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the dosage quotient (DQ) of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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 only valid for germline testing. 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.
- 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 (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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the most genetic alterations in the chromosomal regions (chr 2, 3, 7 and 9) included in this probemix are small (point) mutations, which will not be detected by using SALSA MLPA Probemix P302 Medulloblastoma mix 2.
- 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, 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.

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

Table 1. SALSA MLPA Probemix P302-A3 Medulloblastoma mix 2

Length (nt)	SALSA MLPA probe	Reference	Chromosomal position (hg18)			
			Chr 2	Chr 3	Chr 7	Chr 9
64-105	Control fragments – see table in probemix content section for more information					
121	Reference probe S0864-L25602	21q22				
126	**ATG4B probe** 18123-L25601		2**q**37.3			
131	**TMEM18 probe** 06296-L25684		2p25.3			
137	**BMPR2 probe** 04004-L03427		2**q**33.1			
142	Reference probe 08143-L08022	5p12				
148	**IL1RN probe** 01111-L25685		2**q**13			
154	**EHMT1 probe** 05058-L07382					9**q**34.3
160	**CDKN2A probe** 01524-L13846					9p21.3
166	**CASR probe** 05705-L05223			3**q**13.33		
172	**PTPRD probe** 08332-L08201					9p24.1
178	Reference probe 04857-L04241	5p13				
184	**ROBO1 probe** 06445-L05971			3p12.3		
190	**NBAS probe** 08317-L08186		2p24.3			
196	**PPARG probe** 06906-L14736			3p25.1		
201	**RPRM probe** 10221-L14872		2**q**23.3			
208	**SLITRK3 probe** 10223-L10704			3**q**26.1		
215	**TRPM3 probe** 10225-L14873					9**q**21.11
220	**CDK6 probe** 03183-L02522				7**q**21.2	
226	**DEC1 probe** 10240-L04097					9**q**33.1
236 *	Reference probe 09100-L24261	4q25				
242 ¥	**SHH probe** 06357-L32157				7**q**36.3	
247	**EGFR probe** 05959-L05376				7p11.2	
256 *	Reference probe 17288-L20748	5q14				
265	**CRBN probe** 06311-L05834			3p26.3		
274	**CDKN2A probe** 01291-L00835					9p21.3
283 #	**RPIA probe** 05713-L05151		2p11.2			
294	Reference probe 13579-L23178	19p13				
301	**ZIC1 probe** 08540-L08541			3**q**24		
312	**RTN4 probe** 10238-L09340		2p16.1			
319	Reference probe 06702-L09985	4p16				
329	**ALK probe** 08322-L08191		2p23.2			
337	**IGFBPL1 probe** 05724-L05163					9p13.1
346	**ELN probe** 01335-L00879				7**q**11.23	
355	Reference probe 06711-L06315	15q24				
364	**MAFK probe** 12623-L13707				7p22.3	
373	Reference probe 09779-L10194	15q15				
382	**ALDOB probe** 08668-L08678					9**q**31.1
391	**CDKN2B probe** 10239-L03851					9p21.3
400	**RASSF1 probe** 03991-L03258			3p21.31		
409 *	Reference probe 09720-L32156	12q24				
418	**MCCC1 probe** 06535-L06093			3**q**27.1		
427	**IMPDH1 probe** 06986-L06588				7**q**32.1	
436	**MYCN probe** 03327-L02466		2p24.3			
445	**GHRHR probe** 07216-L06866				7p15.1	
454	**CDKN2B probe** 01531-L00954					9p21.3
463 *	Reference probe 10685-L31869	6p12				
475	**CTNNB1 probe** 03984-L03251			3p22.1		
481 ¥	Reference probe 08614-L32159	12p12				
490	**RELN probe** 10218-L10698				7**q**22.1	
500	Reference probe 17001-L22947	20q11				

* New in version A3.

¥ Changed in version A3. Minor alteration, no change in sequence detected.

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.

Table 2a. P302-A3 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Partial sequence+ (24 nt adjacent to ligation site)	Distance to next probe
Chromosome 2					
131	06296-L25684	TMEM18	2p25.3	TCCTCACCTGCT-TGTCCTCCCGAA	14,6 Mb
190	08317-L08186	NBAS	2p24.3	GTCCCTCCTGCT-TCCATCTCTGAA	766,7 **k**b
436	03327-L02466	MYCN	2p24.3	TGCACCCCCACA-GAAGAAGATAAA	13,8 Mb
329	08322-L08191	ALK	2p23.2	ATCTCACCTGGA-TAATGAAAGACT	25,3 Mb
312	10238-L09340	RTN4	2p16.1	CTGGAGAGACAT-TAAGAAGACTGG	33,7 Mb
283 #	05713-L05151	RPIA	2p11.2	TGGTTCTACAAT-TGTCCATGCTGT	24,8 Mb
148	01111-L25685	IL1RN	2**q**13	CACTGACCTGAG-CGAGAACAGAAA	40,4 Mb
201	10221-L14872	RPRM	2**q**23.3	GAGTGACCTGTT-AAAAGCCACGCA	48,9 Mb
137	04004-L03427	BMPR2	2**q**33.1	ATTTCTTTTCTT-TGCCCTCCTGAT	39,3 Mb
126	18123-L25601	ATG4B	2**q**37.3	AGCGTTCCCTGT-GCAGGCGCCACT	-
Chromosome 3					
265	06311-L05834	CRBN	3p26.3	GATCTGCTCTGT-TGCCCACGATCC	9,3 Mb
196	06906-L14736	PPARG	3p25.1	AACTCTCCTCAA-ATATGGAGTCCA	28,8 Mb
475	03984-L03251	CTNNB1	3p22.1	CTGGCAGCAACA-GTCTTACCTGGA	9,1 Mb
400	03991-L03258	RASSF1	3p21.31	TCCTGCAGAAGT-ACTCCTATTGCC	28,4 Mb
184	06445-L05971	ROBO1	3p12.3	CATCAGTCCACT-GCCACTCTGACT	44,6 Mb
166	05705-L05223	CASR	3**q**13.33	CAGGCAACGCTT-GACCTGAGTCTT	25,2 Mb
301	08540-L08541	ZIC1	3**q**24	TAGCATAGAGGA-ATGTGAGCGCCA	17,8 Mb
208	10223-L10704	SLITRK3	3**q**26.1	GTCTGACTCTGA-GGTAGAGGCTAG	17,8 Mb
418	06535-L06093	MCCC1	3**q**27.1	GTGCTCAGGCCA-ACAGACACACTC	-
Chromosome 7					
364	12623-L13707	MAFK	7p22.3	CACCATCGTCAA-GTCCACCGAGCT	29,4 Mb
445	07216-L06866	GHRHR	7p15.1	GTGGACTCCAGT-GGCGTGATGAGG	24,2 Mb
247	05959-L05376	EGFR	7p11.2	CCGAGGCAGGGA-ATGCGTGGACAA	17,9 Mb
346	01335-L00879	ELN	7**q**11.23	ACCTCATCAACG-TTGGTGCTACTG	19,1 Mb
220	03183-L02522	CDK6	7**q**21.2	GACTTTCTTCAT-TCACACCGAGTA	10,9 Mb
490	10218-L10698	RELN	7**q**22.1	GGGCTATTGATG-AGATTATCATGA	24,8 Mb
427	06986-L06588	IMPDH1	7**q**32.1	CCTCCTAGAACT-ATCTTCAGTGGT	27,5 Mb
242	06357-L32157	SHH	7**q**36.3	CGAGCGATTTAA-GGAACTCACCCC	-
Chromosome 9					
172	08332-L08201	PTPRD	9p24.1	CACAAGGGAGCA-TCATACGTCTTC	13,5 Mb
274	01291-L00835	CDKN2A	9p21.3	TGAAAGAACCAG-AGAGGCTCTGAG	27,1 **k**b
160	01524-L13846	CDKN2A	9p21.3	AAGCGCTCAGAT-GCTCCGCGGCTG	5,3 **k**b
454	01531-L00954	CDKN2B	9p21.3	CCTAGGAAAGGT-GATAGAGCTTAG	8,3 **k**b
391	10239-L03851	CDKN2B	9p21.3	CCTGGAAGCCGG-CGCGGATCCCAA	16,4 Mb
337	05724-L05163	IGFBPL1	9p13.1	GTCAAATAACGG-ATCTTTGTGCTT	34,0 Mb
215	10225-L14873	TRPM3	9**q**21.11	TATGCTGGTGGT-TCTGATGAGCTT	30,8 Mb
382	08668-L08678	ALDOB	9**q**31.1	TTGCAGGGCTTG-ATGGCCTCTCAG	13,8 Mb
226	10240-L04097	DEC1	9**q**33.1	ACTCGCCGCATG-ACCTTGGGAAAA	22,8 Mb
154	05058-L07382	EHMT1	9**q**34.3	GGACCCCGTTGA-TGGAAGCAGCCG	-

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.

Table 2b. Reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Partial sequence+ (24 nt adjacent to ligation site)	Distance to next probe
319	06702-L09985	WFS1	4p16	AGATGGAGGGGC-GCAGCCAGGCCC	104,6 Mb
236	09100-L24261	CFI	4q25	TGTGTGCAACTA-ACAGGAGAAGCT	-
178	04857-L04241	NIPBL	5p13	CTGCAATGTTGC-AAAAATCCTAGA	7,3 Mb
142	08143-L08022	FGF10	5p12	GATGCTGCCAAT-TCAAGGTTTGTG	45,6 Mb
256	17288-L20748	ADGRV1	5q14	CTCATAATTCCA-GTAGTTCGTGGA	-
463	10685-L31869	PKHD1	6p12	TCTGGCATCTAT-ATCTGCAGTCCC	-
481	08614-L32159	H2AFJ	12p12	ACTCAGGACCAA-GTTCTGGGAAGA	101,4 Mb
409	09720-L32156	NOS1	12q24	AGAATATGACAT-TGTGCACCTGGA	-
373	09779-L10194	SPG11	15q15	CCAGTGTAAGCA-GTATGCTATTGG	27,8 Mb
355	06711-L06315	HEXA	15q24	AGGCACTCCACT-TCCTCCTCGAGC	-
294	13579-L23178	CACNA1A	19p13	TGCATCGTCCTC-GCACTGGAGCAG	-
500	17001-L22947	SAMHD1	20q11	CCCTGTCACCTC-AAGTTTGAGGAT	-
121	S0864-L25602	KCNJ6	21q22	AGCTCCTACATC-ACCAGTGAGATC	-

+ Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- **P301 Medulloblastoma mix 1:** Contain probes for chromosomes 6, 14q, 16 and 17.
- **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 copy number changes in neuroblastoma tumours (chromosomes 1, 2, 3, 4, 7, 9, 11, 12, 14 and 17).

References

- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Taylor MD et al. (2012). Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol.* 123:465-72.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P302 Medulloblastoma mix 2

- Gessi M et al. (2013). H3.3 G34R mutations in pediatric primitive neuroectodermal tumors of central nervous system (CNS-PNET) and pediatric glioblastomas: possible diagnostic and therapeutic implications? *J Neurooncol.* 112:67-72.
- Gessi M et al. (2014). MYCN amplification predicts poor outcome for patients with supratentorial primitive neuroectodermal tumors of the central nervous system. *Neuro Oncol.* 16:924-32.

P302 Product history	
Version	*Modification*
A3	Four reference probes are replaced, and several probes have a change in length but no change in the sequence targeted.
A2	Two reference probes included and two reference probes replaced. Control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description
Version A3-01 – 24 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. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

More information: www.mlpa.com; www.mlpa.eu	
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