Product Description

SALSA® MLPA® Probemix P303-A3 Medulloblastoma mix 3

To be used with the MLPA General Protocol.

Version A3
For complete product history see page 9.

This product 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

- **P303-025R:** SALSA® MLPA® Probemix P303 Medulloblastoma mix 3, 25 reactions
- **P303-050R:** SALSA® MLPA® Probemix P303 Medulloblastoma mix 3, 50 reactions
- **P303-100R:** SALSA® MLPA® Probemix P303 Medulloblastoma mix 3, 100 reactions

SALSA® MLPA® Probemix P303 Medulloblastoma mix 3 (hereafter: P303 Medulloblastoma mix 3) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P303-025R	**P303-050R**	**P303-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding 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
SALSA® MLPA® Probemix P303 Medulloblastoma mix 3 is a **research use only (RUO)** assay for the detection of deletions or gains in chromosomes 1, 4q, 5q, 8, 10 and 20, 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 product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Probemix content
P303-A3 Medulloblastoma mix 3 contains 43 MLPA probes with amplification products between 121 and 495 nucleotides (nt). This includes 31 probes for chromosomes 1, 4q, 5q, 8, 10 and 20. In addition, 12 reference probes are included that target relatively copy number stable regions in various cancer types, including medulloblastoma. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com) and in Table 3.

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 MLPA (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 using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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 formalin-fixed, paraffin-embedded (FFPE) tissues, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived 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 described in the table below from the Coriell Institute have been tested with P303-A3 Medulloblastoma mix 3 at MRC Holland and can be used as a positive control samples to detect copy number alterations. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

Sample name	Chromosomal position of CNA (hg18)*	Altered target genes in P303-A3	Expected CNA
Germline samples from Coriell Institute			
NA22995	1p36.32	*TP73*	Heterozygous deletion
NA00214	1q32.1	*KIF14*	Heterozygous deletion
NA17941	1q32.1-q44	*KIF14, LIN9, SH3BP5L*	Heterozygous duplication
NA05347	1q42.12-q44	*LIN9, SH3BP5L*	Heterozygous duplication
NA00782	4q13.2	*GNRHR*	Heterozygous duplication
NA14234	5q22.2	*APC*	Heterozygous deletion
NA10932	8p23.1	*PINX1*	Heterozygous deletion
NA02030	8p23.1-q24.3	*PINX1, GNHR1, FGFR1, CHD7, MYC, PTP4A3*	Heterozygous duplication
NA14485	8p12-p21.2	*GNRH1, FGFR1*	Heterozygous duplication
NA03999	8q24.21	*MYC*	Heterozygous deletion
NA20263	8q24.3	*PTP4A3*	Heterozygous duplication
NA21877	10p12.31	*NEBL*	Heterozygous deletion
NA06936	10p15.1	*KLF6*	Heterozygous deletion
NA20125	10q23.31-q26.13	*PTEN, SUFU, MXI1, DMBT1*	Heterozygous duplication
NA00959	10q24.32-q26.13	*SUFU, MXI1, DMBT1*	Heterozygous duplication
NA05299	10q26.13	*DMBT1*	Heterozygous deletion
NA00981	20p11.21-p12.2	*JAG1, PYGB*	Heterozygous duplication
NA10608	20p12.2	*JAG1*	Heterozygous deletion
NA07945	20q11.23	*NNAT*	Heterozygous deletion

Sample name	Chromosomal position of CNA (hg18)*	Altered target genes in P303-A3	Expected CNA
Cancer cell line sample from Leibniz Institute DSMZ			
HD-MB03 (ACC-740)	1q32.1-q42.12	*KIF14, LIN9*	Gain
	8q24.21	*MYC*+	Gain+

* 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 P303-A3 Medulloblastoma mix 3.
+ High signal, indicating amplification, was observed for S0247-L25707 probe targeting *MYC* gene.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 this criterion is 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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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 *TP73* gene. 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.

P303 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 alterations are small (point mutations), which will not be detected using P303-A3 Medulloblastoma mix 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, 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 in sequence data 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
https://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 (e.g., a gain of *KIF14* and *SH3BP5L* but not *LIN9)* to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P303-A3 Medulloblastoma mix 3

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)						Location (hg18) in kb
		Reference	Chr 1	Chr 8	Chr 10	Chr 20	Other	
64-105	Control fragments – see table in probemix content section for more information							
121	Reference probe S0864-L25602	21q22						21-037,920
126	**MYC probe** S0247-L25707			8**q**24.21				08-128,822
132 *	CD160 probe 22652-L32135		1**q**21.1					01-144,415
136	**PDGFRA probe** 10247-L10719						4**q**12	04-054,822
142	Reference probe 08143-L08022	5p12						05-044,337
154	**NNAT probe** 05213-L05461					20**q**11.23		20-035,583
160	Reference probe 12741-L13835	21q22						21-042,050
174	**AURKA probe** 08609-L08621					20**q**13.2		20-054,390
178	**PTEN probe** 03636-L06030				10**q**23.31			10-089,675
184	**ADGRV1** probe 03994-L03261						5**q**14.3	05-090,495
190 «	**TP73 probe** 10241-L01262		1p36.32					01-003,558
196	Reference probe 09580-L10629	22q13						22-036,841
203	**DMBT1 probe** 08228-L07929				10**q**26.13			10-124,311
216	**CHD7 probe** 06746-L25694			8**q**12.2				08-061,883
226	**PTAFR probe** 10242-L01425		1p35.3					01-028,350
236 *	Reference probe 19652-L26684	4p13						04-042,278
247	**MXI1 probe** 10229-L10710				10**q**25.2			10-112,035
258 *	Reference probe 04534-L22019	2q24						02-166,606
265	**SH3BP5L probe** 10243-L02149		1**q**44					01-247,075
274	**JAG1 probe** 05989-L05414					20p12.2		20-010,571
283	Reference probe 08214-L08147	2q14						02-127,901
292 ¥	**LIN9 probe** 21534-L32094		1**q**42.12					01-224,521
301	**PINX1 probe** 10230-L10711			8p23.1				08-010,728
310	**RPE65 probe** 03603-L02970		1p31.3					01-068,677
319	**FGFR1 probe** 04438-L03824			8p12				08-038,395
328	**APC probe** 01774-L01340						5**q**22.2	05-112,192
338 *	Reference probe 12785-L15496	2q13						02-108,972
346	**ZNF25 probe** 06709-L06296				10p11.21			10-038,281
364 ¥	Reference probe 06711-L32142	15q24						15-070,455
373 ¥	**KIF14 probe** 21535-L32093		1**q**32.1					01-198,851
382	**KLF6 probe** 06672-L06245				10p15.1			10-003,808
391	**NTNG1 probe** 06482-L06008		1p13.3					01-107,669
402	**PYGB probe** 05740-L05179					20p11.21		20-025,198
409	**MYOT probe** 11561-L25695						5**q**31.2	05-137,246
418	**SUFU probe** 10235-L10716				10**q**24.32			10-104,380
427	**GNRH1 probe** 04444-L03830			8p21.2				08-025,333
436 ¥	**PTP4A3 probe** 22749-L32092			8**q**24.3				08-142,510
445	**GNRHR probe** 04448-L03834						4**q**13.2	04-068,289
454	Reference probe 07607-L07292	15q26						15-097,300
463 *	Reference probe 10685-L31869	6p12						06-051,876
472	**PTCH2 probe** 10232-L10713		1p34.1					01-045,068
483	**NEBL probe** 08480-L08491				10p12.31			10-021,226
495	Reference probe 16456-L25697	18q21						18-045,630

* New in version A3.
¥ Changed in version A3. 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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

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

Table 2. P303-A3 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location (hg18)	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
Chromosome 1					
190 «	10241-L01262	*TP73*	1p36.32	GAGACCCGGGTG-TCAGGAAAGATG	24,8 **M**b
226	10242-L01425	*PTAFR*	1p35.3	CATCTTCATCGT-GTTCAGCTTCTT	16,7 **M**b
472	10232-L10713	*PTCH2*	1p34.1	CCAGCAGATCCA-TGCCTTCTCCTC	23,6 **M**b
310	03603-L02970	*RPE65*	1p31.3	TCTTCCATCACA-TCAACACCTATG	39,0 **M**b
391	06482-L06008	*NTNG1*	1p13.3	ATCACTCTGTCT-TGGAGCAAAACC	36,7 **M**b
132	22652-L32135	*CD160*	1**q**21.1	CCATAAGCCAAG-TCACACCGTTGC	54,4 **M**b
373	21535-L32093	*KIF14*	1**q**32.1	ACCACTCCTAGA-AAGAGCCTTCGA	25,7 **M**b
292	21534-L32094	*LIN9*	1**q**42.12	GGCCTTCTCGAT-TTTTTATGACCC	22,6 **M**b
265	10243-L02149	*SH3BP5L*	1**q**44	GAGAAATGGTGT-TTGTGGCTGAGC	-
Chromosome 4q					
136	10247-L10719	*PDGFRA*	4**q**12	GGAGAGTGAAGT-GAGCTGGCAGTA	13,5 **M**b
445	04448-L03834	*GNRHR*	4**q**13.2	TCTAAAAATGAC-GGTTGCATTTGC	-
Chromosome 5q					
184	03994-L03261	*ADGRV1*	5**q**14.3	TCAGTGATAATG-AATCTGGTCAAG	21,7 **M**b
328	01774-L01340	*APC*	5**q**22.2	CAGATTTTAGTT-GGGCCACAAGTG	25,1 **M**b
409	11561-L25695	*MYOT*	5**q**31.2	ACCCACCACGTT-TCATTCAAGTGC	-
Chromosome 8					
301	10230-L10711	*PINX1*	8p23.1	GCTACCATCAAT-AATGAAGTGAGC	14,6 **M**b
427	04444-L03830	*GNRH1*	8p21.2	AGAAGATTTAAA-TCCATTGGGCCA	13,1 **M**b
319	04438-L03824	*FGFR1*	8p12	TCCATGAACTCT-GGGGTTCTTCTG	23,5 **M**b
216	06746-L25694	*CHD7*	8**q**12.2	ATCTGGAGAGGA-GGTAGAAATTGA	66,9 **M**b
126	S0247-L25707	*MYC*	8**q**24.21	TCTTAAAGAGGA-GGAACAAGAAGA	13,7 **M**b
436	22749-L32092	*PTP4A3*	8**q**24.3	CACACACGCACA-AGACCCGGTGCT	-
Chromosome 10					
382	06672-L06245	*KLF6*	10p15.1	TGAATGCATCAA-ATACTCTTCTCC	17,4 **M**b
483	08480-L08491	*NEBL*	10p12.31	CTGGGATCCTTT-TCTGTTCACTCA	17,1 **M**b
346	06709-L06296	*ZNF25*	10p11.21	CTTGACTGAATT-AATCCTTCAAAA	51,4 **M**b
178	03636-L06030	*PTEN*	10**q**23.31	TTGATCTGCTTT-AAATGACTTGGC	14,7 **M**b
418	10235-L10716	*SUFU*	10**q**24.32	GGCCTGAAAAGA-AGCTGAAGGTCT	7,7 **M**b
247	10229-L10710	*MXI1*	10**q**25.2	TCCCATGGAGAA-GTGGACAATATA	12,3 **M**b
203	08228-L07929	*DMBT1*	10**q**26.13	ATGTGGCTTCTT-TCTACAGGTGTG	-
Chromosome 20					
274	05989-L05414	*JAG1*	20p12.2	GGAGCGACCTGT-GTGGATGAGATC	14,6 **M**b
402	05740-L05179	*PYGB*	20p11.21	GAGATAGAAGAA-GATGCTGGCCTT	10,4 **M**b
154	05213-L05461	*NNAT*	20**q**11.23	CCTAAGTGCGCA-TGCGCGCCTTGG	18,8 **M**b
174	08609-L08621	*AURKA*	20**q**13.2	AGGCATCCTAAT-ATTCTTAGACTG	-

« 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.

Table 3. Reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
338	12785-L15496	*EDAR*	2q13	CCCAGAACTGGA-TGGTACCTGACT	18,9 **M**b
283	08214-L08147	*PROC*	2q14	GTCAGGCTTGGT-ATGGGCTGGAGC	38,7 **M**b
258	04534-L22019	*SCN1A*	2q24	ACTGTTCTCCAT-ATTGGTTAAAAG	-
236	19652-L26684	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	12,5 **M**b
142	08143-L08022	*FGF10*	5p12	GATGCTGCCAAT-TCAAGGTTTGTG	46,2 **M**b

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
463	10685-L31869	*PKHD1*	6p12	TCTGGCATCTAT-ATCTGCAGTCCC	-
364	06711-L32142	*HEXA*	15q24	AGGCACTCCACT-TCCTCCTCGAGC	26,8 **M**b
454	07607-L07292	*IGF1R*	15q26	CATGGTAGCCGA-AGATTTCACAGT	-
495	16456-L25697	*MYO5B*	18q21	TCTGACTCATCA-TCTCCCACTTCC	-
121	S0864-L25602	*KCNJ6*	21q22	AGCTCCTACATC-ACCAGTGAGATC	4,1 **M**b
160	12741-L13835	*RIPK4*	21q22	AAGCCAAGAAGA-TGGAGATGGCCA	-
196	09580-L10629	*PLA2G6*	22q13	TCCATGAGTACA-ATCAGGACCTGA	-

Complete probe sequences are available at www.mrcholland.com.

Related products
For related products, see the product page on our website.

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.
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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
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- 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 P303 Medulloblastoma mix 3

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P303 product history	
Version	*Modification*
A3	One flanking probe (1q) and four reference probes have been replaced and three removed. In addition, several probes have a change in length but not in the sequence detected.
A2	Two reference probes included and one reference probe replaced. Control fragments adjusted (QDX2).
A1	First release.

Implemented changes in the product description

Version A3-04 - April 2024 (05P)
- Product description updated to new template.
- "Location (hg 18) in kb" column added in Table 1.
- Added one cancer cell line sample in 'Positive control DNA samples' section.
- Added P303 specific note on page 3.
- Various minor textual or layout changes.

Version A3-03- 01 April 2022 (02P)
- Typographical error corrected on page 1.
- New references added to selected publications using P303 on page 7.

Version A3-02 - 12 January 2021 (02P)
- Table in section Positive control DNA samples on page 2 is corrected (sample NA10932 mentioned once).
- Minor layout change for Table 2a and Table 2b.

Version A3-01 - 25 August 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).

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