Product Description
SALSA® MLPA® Probemix P088-D1 Oligodendroglioma 1p-19q

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 12.

Catalogue numbers:
- **P088-025R:** SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q, 25 reactions.
- **P088-050R:** SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q, 50 reactions.
- **P088-100R:** SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q, 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.

Intended purpose
The SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of co-deletion of chromosome arms 1p and 19q, and deletions of the *CDKN2A* and *CDKN2B* genes, and for detection of the most common somatic point mutations in *IDH1* (p.R132H and p.R132C) and *IDH2* (p.R172K and p.R172M) in genomic DNA isolated from fresh-frozen or formalin-fixed paraffin-embedded (FFPE) human glioma specimens. P088 Oligodendroglioma 1p-19q is intended to aid in diagnosis of oligodendroglioma.

Deletions of *CDKN2A* and/or *CDKN2B* detected with P088 Oligodendroglioma 1p-19q should be confirmed with a different technique. In particular, deletions detected by only a single probe always require confirmation by another method. Except for the four mutations mentioned above, no other mutations in *IDH1* and *IDH2* can be detected with P088 Oligodendroglioma 1p-19q. For detection of rare mutations in *IDH1* and *IDH2*, it is recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit, Coffalyser.Net analysis software, and SALSA Binning DNA SD079.

Clinical background
Oligodendrogliomas are a type of glioma (central nervous system neoplasm) that originate from the oligodendrocytes of the brain or from a glial precursor cell. According to the WHO Classification of Tumours of the Central Nervous System, oligodendroglioma is molecularly defined by co-deletion of chromosome arms 1p and 19q in combination with a mutation in *IDH1* or *IDH2* (Louis et al. 2016; Louis et al. 2021). The 1p/19q

co-deletion distinguishes oligodendrogliomas from other gliomas, and is considered an early event in oligodendroglioma tumorigenesis (Pinkham et al. 2015). The co-deletion is thought to be the result of an unbalanced whole-arm translocation between chromosomes 1 and 19 with the loss of the resulting hybrid chromosome (Griffin et al. 2006; Jenkins et al. 2006). The IDH1 p.R132H mutation accounts for ~90% of all *IDH1* and *IDH2* mutations. The remaining ~10% are other substitutions in codon 132 of *IDH1* (e.g. p.R132C, p.R132S, p.R132G) or substitutions in codon 172 of *IDH2* (p.R172K, p.R172M or p.R172W) (Cahill et al. 2015; Hartmann et al. 2009). p.R132H accounts for 92.7% and p.R132C for 4% of all IDH1 mutations, and p.R172K accounts for 64.5% and p.R172M for 19% of all IDH2 mutations (Hartmann et al. 2009).

Gene structure and transcript variants
For information on gene structure and transcript variants of genes targeted by P088-D1 Oligodendroglioma 1p-19q, see http://www.lrg-sequence.org/ and https://www.ncbi.nlm.nih.gov/gene.

Exon numbering
The *IDH1* and *IDH2* exon numbering used in this P088-D1 Oligodendroglioma 1p-19q product description is the exon numbering from the LRG_610 and LRG_611 sequences, respectively. For *CDKN2A* and *CDKN2B*, the exon numbering of the MANE transcripts is used. The *CDKN2A* exon numbering has changed. From description version D1-02 onwards, we have adopted the *CDKN2A* exon numbering of the NM_000077.5 (MANE Select) and NM_058195.4 (MANE Plus Clinical) transcripts. The *CDKN2A* exon numbering used in previous versions of this product description (based on LRG_11) can be found in between brackets in Table 2. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P088-D1 Oligodendroglioma 1p-19q contains 59 MLPA probes with amplification products between 126 and 509 nucleotides (nt). This includes 19 probes for the 1p arm and 12 probes for the 19q arm. In addition, three flanking probes for the 1q arm and two flanking probes for the 19p arm have been included to discriminate between chromosome arm losses and whole chromosome losses. The probemix also contains four probes specific for the p.R132H and p.R132C mutations in *IDH1* and the p.R172K and p.R172M mutations in *IDH2* which will only generate a signal when the mutation is present. Furthermore, the probemix contains three probes for *CDKN2A* and two probes for *CDKN2B*. Finally, 14 reference probes are included that target relatively copy number stable regions in central nervous system tumours, especially in oligodendrogliomas. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com) and in Table 3, respectively.

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 from fresh-frozen or FFPE human glioma specimens, free from impurities known to affect MLPA reactions. A reliable MLPA analysis requires a minimum of 30% tumour cells, but a tumour cell percentage of at least 50% is recommended. Glioma specimens should be evaluated by a pathologist before extraction of DNA. 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 oligodendroglioma and should have a tissue composition similar to that of patient samples. 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 and Leibniz Institute DSMZ described in the table below have been tested with this P088-D1 probemix at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Altered target regions/genes in P088-D1	Expected copy number alteration
NA22976	Coriell Institute	1p36.33-1p36.32 (*GNB1, TNFRSF14* and *TP73*) 9p21.3 (*CDKN2A* and *CDKN2B*)	Heterozygous deletion Heterozygous duplication
CADO-ES1*	Leibniz Institute DSMZ	9p21.3 (*CDKN2A, CDKN2B*) 1q31.3-1q32.1 (*CRB1* and *TNNT2*)	Homozygous deletion Heterozygous duplication
HT-1376◊	Leibniz Institute DSMZ	complete 19q arm 1p34.1-1p33 (*MUTYH*, *PRDX1*, *FAF1*, *CDKN2C*) 1p31.3-1p21.3 (*MIR101*, *FUBP1*, *GTF2B*, *DPYD*)	Heterozygous deletion Heterozygous duplication Heterozygous duplication
LOPRA-1◊	Leibniz Institute DSMZ	complete 1p arm 1p33 *(FAF1, CDKN2C)* 9p21.3 *(CDKN2A, CDKN2B)*	Heterozygous deletion Homozygous deletion Heterozygous deletion

* Some of the reference probe targets are also affected by copy number alterations in this tumour cell line.
◊ Some of the reference probe targets and some target regions/genes not mentioned in the table are also affected by copy number alterations in this tumour cell line.

SALSA Binning DNA SD079
The Binning DNA SD079 provided with this probemix can be used for binning of all probes including the four mutation-specific probes (203 nt probe 19529-L16492 for the IDH1 p.R132H (c.395G>A) mutation, 227 nt probe 14787-L23353 for the IDH1 p.R132C (c.394C>T) mutation, 238 nt probe 20963-L29002 for the IDH2 p.R172K (c.515G>A) mutation, and 244 nt probe 20963-L29001 for the IDH2 p.R172M (c.515G>T) mutation).

Binning DNA SD079 is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequences detected by the above mentioned probes. Inclusion of one reaction with 5 µl Binning DNA SD079 in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the Binning DNA SD079 product description, available online: www.mrcholland.com.

Performance characteristics

Oligodendroglioma is molecularly defined by co-deletion of chromosome arms 1p and 19q in combination with a mutation in *IDH1* or *IDH2* (Louis et al. 2016; Louis et al. 2021). The IDH1 p.R132H mutation accounts for ~90% of all *IDH1* and *IDH2* mutations. The remaining ~10% are other substitutions in codon 132 of *IDH1* (e.g. p.R132C, p.R132S, p.R132G) or substitutions in codon 172 of *IDH2* (p.R172K, p.R172M or p.R172W) (Cahill et al. 2015; Hartmann et al. 2009). The four mutation-specific probes included in P088 Oligodendroglioma 1p-19q can detect 96% of all *IDH1* and *IDH2* mutations in oligodendrogliomas (Hartmann et al. 2009). A good correlation (>97% concordance) between MLPA results and other techniques, including FISH and array CGH, has been reported in literature (based on a 2005-2022 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for probes targeting 1p, 19q, *CDKN2A* and *CDKN2B* are allele copy numbers of 2 (normal), 1 (heterozygous deletion) or 0 (homozygous deletion).

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	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

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

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.

- 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 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 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.

P088 specific notes:

- Detection of 1p/19q co-deletion should be based on the results of all probes on these chromosome arms. Nineteen probes are present for chromosome 1p and twelve probes are present for chromosome 19q, which minimizes the risk of false positive results due to single deviating probe results. When results are inconclusive, follow-up studies are required to confirm the result.
- The presence of a clear signal for a mutation-specific probe, i.e. a signal that is at least 10% of the median signal of the reference probes in that sample AND higher than any background signal observed for the mutation-specific probe in reference samples, indicates that the mutation is present.
- The percentage of tumour cells present in a sample affects the sensitivity of the probemix for mutation detection. Dilution series using a mutation-positive cell line or reference standard have shown that a reliable detection of the *IDH1* and *IDH2* mutations is possible when the allelic burden is at least 12.5%. Note though that sensitivity for mutation detection may vary depending on the sample type and DNA extraction method used.
- Use of FFPE tissues can result in low quality of the extracted DNA due to sample fixation and storage conditions. This might result in higher probe standard deviations. Warnings during the Fragment Analysis using Coffalyser.Net will indicate that the MLPA experiment was not optimal on the specific sample(s) used. For more information on the use of FFPE tissues with MLPA, please refer to Atanesyan et al. (2017).

Limitations of the procedure

- Not all mutations in the *IDH1* and *IDH2* genes will be detected by using SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q. It is therefore recommended to use this assay in combination with sequence analysis.
- 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.

Confirmation of results

Deletions detected by only a single CDKN2A or CDKN2B 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.

Deletions of CDKN2A and/or CDKN2B detected by more than one consecutive probe and inconclusive results for the 1p/19q probes 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.

The presence of the *IDH1* p.R132H, *IDH1* p.R132C, *IDH2* p.R172K and *IDH2* p.R172M mutations can be confirmed by sequence analysis.

Mutation databases

https://www.ncbi.nlm.nih.gov/clinvar/?term=IDH1; https://www.ncbi.nlm.nih.gov/clinvar/?term=IDH2; for all genes: http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in ClinVar and/or the Catalogue Of Somatic Mutations In Cancer (COSMIC). 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 deletion detected by the *CDKN2A* exon 1 and exon 2 probes at 190 nt and 385 nt, respectively, but not by the *CDKN2A* exon 1 probe at 250 nt) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P088-D1 Oligodendroglioma 1p-19q

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					Location (hg18) in kb
		Reference	Chr. 1	Chr. 19	*IDH1/2* mutation	*CDKN2A/B*	
64-105	Control fragments – see table in probemix content section for more information						
126 *	Reference probe 22212-L31462	18p11					18-013,724
131	**NOTCH2 probe** 05745-L05183		1p12				01-120,331
136 * «	**TNFRSF9 probe** 20271-L27994		1p36.23				01-007,923
142	**CDKN2B probe** 11867-L23298					Exon 1	09-021,999
148 ¬	**SMARCA4 probe** 02488-L22890			19**p**13.2			19-011,031
153 ¥	Reference probe 22204-L23302	11q22					11-098,932
157 «	**UPK1A probe** 18116-L23103			19q13.12			19-040,856
163	**PTAFR probe** 18115-L23104		1p35.3				01-028,350
167 «	**CCNE1 probe** 02881-L23105			19q12			19-035,005
172	Reference probe 15449-L23605	12q13					12-046,676
178	**GNB1 probe** 02890-L20648		1p36.33				01-001,747
184	**PDCD5 probe** 02882-L02349			19q13.11			19-037,764
190	**CDKN2A probe** 16880-L20211					Exon 1	09-021,984
196 ¬	**TNNT2 probe** 06557-L20938		1**q**32.1				01-199,604
203 §	**IDH1 probe** 19529-L16492				p.R132H		02-208,821
208	Reference probe 16261-L18553	20q11					20-034,979
214 ¬	**LDLR probe** 02314-L20213			19**p**13.2			19-011,077
220	**PPP1R15A probe** 02887-L02354			19q13.33			19-054,070
227 § +	**IDH1 probe** 14787-L23353				p.R132C		02-208,821
232 *	Reference probe 22205-L31582	3p12					03-081,667
238 §	**IDH2 probe** 20963-L29002				p.R172K		15-088,433
244 §	**IDH2 probe** 20963-L29001				p.R172M		15-088,433
250 ¥	**CDKN2A probe** 16060-L19714					Exon 1	09-021,965
255 ¥	Reference probe 22209-L31583	17p13					17-007,355
259 ¥	**GTF2B probe** 02871-L31584		1p22.2				01-089,126
265 *	**SLC7A9 probe** 17872-L31766			19q13.11			19-038,013
272 ¥	**BAX probe** 22201-L31777			19q13.33			19-054,151
276	Reference probe 17450-L29159	16p13					16-009,761
282 «	**CDKN2C probe** 18565-L24220		1p33				01-051,208
288	**FAF1 probe** 02877-L24219		1p33				01-051,026
293 «	**WNT4 probe** 06055-L24329		1p36.12				01-022,329
299 *	Reference probe 22210-L25962	6q21					06-108,321
306 ¬	**LMNA probe** 16877-L19710		1**q**22				01-154,372
313 «	**CHMP2A probe** 18119-L29136			19q13.43			19-063,757
319	Reference probe 04833-L22803	5p13					05-037,032
325 «	**CHMP2A probe** 18118-L23300			19q13.43			19-063,755
332 «	**TP73 probe** 01682-L24330		1p36.32				01-003,558
340 ¥ «	**MIR101-1 probe** 13654-L31465		1p31.3				01-065,297
346	**TNFRSF14 probe** 04693-L24421		1p36.32				01-002,480
355	Reference probe 06426-L05952	6p22					06-024,386
362	**TGFB1 probe** 02889-L23352			19q13.2			19-046,542
370	**FUBP1 probe** 18571-L24211		1p31.1				01-078,203
377 «	**DPYD probe** 02870-L23108		1p21.3				01-098,159
385 ¥	**CDKN2A probe** 22202-L23102					Exon 2	09-021,961
392 *	**WDR62 probe** 22375-L31560			19q13.12			19-041,287
399 ¥	Reference probe 22206-L23109	2p11					02-085,640
408 ¥ «	**CDKN2C probe** 21384-L31263		1p33				01-051,212
412	**MFN2 probe** 20882-L29180		1p36.22				01-011,984
420 «	**ZNF296 probe** 03221-L24213			19q13.32			19-050,271
427 ¬	**CRB1 probe** 06961-L24214		1**q**31.3				01-195,593

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					Location (hg18) in kb
		Reference	Chr. 1	Chr. 19	*IDH1/2* mutation	*CDKN2A/B*	
436	Reference probe 10634-L11182	8q12					08-061,856
445	**PLPP3 probe** 18120-L24277		1p32.2				01-056,775
454 *	**MUTYH probe** 22208-L30955		1p34.1				01-045,571
463 «	**CIC probe** 18575-L24215			19q13.2			19-047,487
474	**NRAS probe** 01032-L20220		1p13.2				01-115,053
481	Reference probe 09772-L10187	15q21					15-042,706
490 ¥	**CDKN2B probe** 22203-L23606					Exon 2	09-021,996
498 ¥	**PRDX1 probe** 18413-L31464		1p34.1				01-045,760
509 *	Reference probe 22207-L31264	5q14					05-090,048

[a] See section Exon numbering on page 2 for more information.

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present.
+ A small background signal may be observed for this probe in samples without the IDH1 p.R132C mutation.
« 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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. P088-D1 target probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
Chromosome 1 Co-deletion of the 1p and 19q chromosome arms is a diagnostic molecular marker in oligodendroglioma (Louis et al. 2016; Louis et al. 2021). Probes for the 1q chromosome arm enable discrimination between loss of a chromosome arm and loss of the complete chromosome.						
1**p** arm						
178	02890-L20648	***GNB1***	1p36.33	CTAAGATCGGAA-GATGAGTGAGCT	0.7 Mb	01-001,747
346	04693-L24421	***TNFRSF14***	1p36.32	CAATACCCTCAT-TCACGGGGAGGA	1.1 Mb	01-002,480
332 «	01682-L24330	***TP73***	1p36.32	GAGACCCGGGTG-TCAGGAAAGATG	4.4 Mb	01-003,558
136 «	20271-L27994	***TNFRSF9***	1p36.23	GAAGACCAAGGA-GTGGAAAGTTCT	4.1 Mb	01-007,923
412	20882-L29180	***MFN2***	1p36.22	CGCAGAAGGCTT-TCAAGTGAGGAT	10.3 Mb	01-011,984
293 «	06055-L24329	***WNT4***	1p36.12	GCGAGAAACTCA-AGGGCCTGATCC	6.0 Mb	01-022,329
163	18115-L23104	***PTAFR***	1p35.3	TGCCCGCCTGTA-CCCTTGCAAGAA	17.2 Mb	01-028,350
454	22208-L30955	***MUTYH***	1p34.1	CATTGGTGCTGA-TCCCAGCAGCAC	0.2 Mb	01-045,571
498	18413-L31464	***PRDX1***	1p34.1	ACCTCAGCCATC-CGCAACAGGGTG	5.3 Mb	01-045,760
288	02877-L24219	***FAF1***	1p33	GGACCTGCATTT-AATCCAGCAAGT	0.2 Mb	01-051,026
282 «	18565-L24220	***CDKN2C***	1p33	CCGGAGGGTTAA-AAGATGATCGCC	4.3 **k**b	01-051,208
408 «	21384-L31263	***CDKN2C***	1p33	TGCTGGAGTTTC-AAGCTGATGTTA	5.6 Mb	01-051,212
445	18120-L24277	***PLPP3***	1p32.2	AGCACCATCAAG-CCTTACCACCGA	8.5 Mb	01-056,775
340 «	13654-L31465	***MIR101-1***	1p31.3	GGATGGCAGCCA-TCTTACCTTCCA	12.9 Mb	01-065,297
370	18571-L24211	***FUBP1***	1p31.1	CCATCATGGCGA-TGGACCGGGAAA	10.9 Mb	01-078,203
259	02871-L31584	***GTF2B***	1p22.2	CAGATGCGATTT-TAGTGGAGGACT	9.0 Mb	01-089,126
377 «	02870-L23108	***DPYD***	1p21.3	CTGCTGTCACTT-GGCTCTCTGGCT	16.9 Mb	01-098,159
474	01032-L20220	***NRAS***	1p13.2	TGATGGGACTCA-GGGTTGTATGGG	5.3 Mb	01-115,053
131	05745-L05183	***NOTCH2***	1p12	GGGGTCAACACT-TACAACTGCCGC	34.0 Mb	01-120,331

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
1**q** arm						
306 ¬	16877-L19710	*LMNA*	1q22	ACTGCCTGGCAT-TGTCCAGCTGGA	41.2 Mb	01-154,372
427 ¬	06961-L24214	*CRB1*	1q31.3	GGAATGTGTGGA-GCTGTCCTCAGA	4.0 Mb	01-195,593
196 ¬	06557-L20938	*TNNT2*	1q32.1	TTTGCTTCCTCT-TCTTCTTCATCT	-	01-199,604
IDH1 gene, 2q33.3. Mutation of *IDH1* is a diagnostic molecular marker in both oligodendroglioma and astrocytoma. The p.R132H (c.395G>A) and p.R132C (c.394C>T) mutations have been detected by sequencing in 664 samples (92.7% of all *IDH1* mutations) and 29 samples (4.2% of all *IDH1* mutations), respectively, in a cohort of 1,010 diffuse gliomas that included oligodendrogliomas, astrocytomas and oligoastrocytomas (Hartmann et al. 2009). Ligation sites are indicated according to NM_005896.4.						
203 §	19529-L16492	***IDH1***, exon 6; **p.R132H** (c.395G>A)	618-619	CATCATAGGTC**A**-TCATGCTTATGG	-	02-208,821
227 § +	14787-L23353	***IDH1***, exon 6; **p.R132C** (c.394C>T)	617-616, reverse	ATAAGCATGAC**A**-ACCTATGATGAT	-	02-208,821
CDKN2A/B genes, 9p21.3. Deletions of *CDKN2A* and *CDKN2B* are found in a subset of oligodendrogliomas. Homozygous deletion of *CDKN2A* is thought to be associated with a worse patient prognosis and malignant progression (Appay et al. 2019; Bigner et al. 1999; Michaud et al. 2016), but is not yet considered a consistent marker to predict poor outcome in oligodendroglioma (Komori 2022). Deletions of *CDKN2A* and *CDKN2B* are not unique to oligodendroglioma. *CDKN2A/B* homozygous deletion is an important determinant in the grading of IDH-mutant astrocytoma, with presence of *CDKN2A/B* homozygous deletion resulting in a WHO grade 4 classification (Louis et al. 2021). Additionally, homozygous deletion of *CDKN2A* has been reported to define a subset of malignant astrocytomas in children (Schiffman et al. 2010). For *CDKN2A*, exon numbering and ligation sites are according to NM_000077.5 (MANE Select transcript; $p16^{INK4a}$) and NM_058195.4 (MANE Plus Clinical transcript; $p14^{ARF}$) as indicated. For *CDKN2B*, exon numbering and ligation sites are according to NM_004936.4. In case of apparent deletions, SALSA MLPA Probemix P419 CDKN2A/2B-CDK4 can be used to determine the extent of the deletion.						
385	22202-L23102	***CDKN2A***, exon 2 (3)	NM_000077.5: 45 nt before exon 2	TCCTTTCCGTCA-TGCCGGCCCCCA	3.7 **kb**	09-021,961
250	16060-L19714	***CDKN2A***, exon 1 (2)	NM_000077.5: 138 nt before exon 1	GCCTGGAAAGAT-ACCGCGGTCCCT	19.4 **kb**	09-021,965
190	16880-L20211	***CDKN2A***, exon 1	NM_058195.4: 23 nt before exon 1	AGTCTGCAGTTA-AGGGGGCAGGAG	11.4 **kb**	09-021,984
490	22203-L23606	***CDKN2B***, exon 2	899-900	GCCTGTCTGAGA-CTCACAGGAAGG	3.1 **kb**	09-021,996
142	11867-L23298	***CDKN2B***, exon 1	319-320	CCAACGGTGGAT-TATCCGGGCCGC	-	09-021,999
IDH2 gene, 15q26.1. Mutation of *IDH2* is a diagnostic molecular marker in both oligodendroglioma and astrocytoma. The p.R172K (c.515G>A) and p.R172M (c.515G>T) mutations have been detected by sequencing in 20 samples (64.5% of all *IDH2* mutations) and 6 samples (19.3% of all *IDH2* mutations), respectively, in a cohort of 1,010 diffuse gliomas that included oligodendrogliomas, astrocytomas and oligoastrocytomas (Hartmann et al. 2009). Ligation sites are indicated according to NM_002168.4.						
238 §	20963-L29002	***IDH2***, exon 5; **p.R172K** (c.515G>A)	593-594	TACCATTGGCA**A**-GCACGCCCATGG	-	15-088,433
244 §	20963-L29001	***IDH2***, exon 5; **p.R172M** (c.515G>T)	593-594	TACCATTGGCA**T**-GCACGCCCATGG	-	15-088,433
Chromosome 19 Co-deletion of the 19q and 1p chromosome arms is a diagnostic molecular marker in oligodendroglioma (Louis et al. 2016; Louis et al. 2021). Probes for the 19p chromosome arm enable discrimination between loss of a chromosome arm and loss of the complete chromosome.						
19**p** arm						
148 ¬	02488-L22890	*SMARCA4*	19p13.2	CGTCTTGCAGTC-GGTCTTCACCAG	45.5 **kb**	19-011,031

Length (nt)	SALSA MLPA probe	Gene/Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe	Location (hg18) in kb
214 ¬	02314-L20213	*LDLR*	19p13.2	TCTGTGACTCAG-ACCGGGACTGCT	23.9 Mb	19-011,077
19**q** arm						
167 «	02881-L23105	***CCNE1***	19q12	GATGGTTCCATT-TGCCATGGTTAT	2.8 Mb	19-035,005
184	02882-L02349	***PDCD5***	19q13.11	CGAGGAGCTTGA-GGCGCTGAGGAG	0.2 Mb	19-037,764
265	17872-L31766	***SLC7A9***	19q13.11	GATGCTAATGGA-AGTGGTCCCACC	2.8 Mb	19-038,013
157 «	18116-L23103	***UPK1A***	19q13.12	GATGGTGTCCAA-CCCATCCCTGAT	0.4 Mb	19-040,856
392	22375-L31560	***WDR62***	19q13.12	GGCTGCAGACCA-CCTTCCAAGAAG	5.3 Mb	19-041,287
362	02889-L23352	***TGFB1***	19q13.2	GAGTGGTTATCT-TTTGATGTCACC	0.9 Mb	19-046,542
463 «	18575-L24215	***CIC***	19q13.2	GAAACATCCTGC-AGACACTGGTGC	2.8 Mb	19-047,487
420 «	03221-L24213	***ZNF296***	19q13.32	TCATGGACCACA-AGAAGCTGGGCT	3.8 Mb	19-050,271
220	02887-L02354	***PPP1R15A***	19q13.33	GATGTGGATAGT-GAGGATAAGGAA	81.7 **k**b	19-054,070
272	22201-L31777	***BAX***	19q13.33	TCCCCCCGAGAG-GTCTTTTTCCGA	9.6 Mb	19-054,151
325 «	18118-L23300	***CHMP2A***	19q13.43	TGGAGTTTGAGC-GGCAGGCAGAGA	2.0 **k**b	19-063,755
313 «	18119-L29136	***CHMP2A***	19q13.43	GGGCCCTGAACC-GTGCCATGCGGG	-	19-063,757

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present.
+ A small background signal may be observed for this probe in samples without the IDH1 p.R132C mutation.
« 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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. P088-D1 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	Location (hg18) in kb
399	22206-L23109	*GGCX*	2p11	CACCATCATGTT-TCTGGGTGAGGG	123 Mb to *IDH1*	02-085,640
232	22205-L31582	*GBE1*	3p12	ACCGAGTTGGAA-CAGCATTGCCAG	-	03-081,667
319	04833-L22803	*NIPBL*	5p13	ACGTGTGAAAAT-GAACAAACGCAA	53.0 Mb	05-037,032
509	22207-L31264	*ADGRV1*	5q14	GTTCGGGAACCT-GCACAAGGATTG	-	05-090,048
355	06426-L05952	*DCDC2*	6p22	TTTAGGGAAATG-ATCGCCACTCTA	83.9 Mb	06-024,386
299	22210-L25962	*SEC63*	6q21	CAGCAGGGTGAA-ACTAACAAGAAC	-	06-108,321
436	10634-L11182	*CHD7*	8q12	GGATCCCAGTAA-AGGTTTTGGTAA	-	08-061,856
153	22204-L23302	*CNTN5*	11q22	CACCAGAGCTGT-TAAACACATTGA	-	11-098,932
172	15449-L23605	*COL2A1*	12q13	CTGGTATCCTCA-TTTTACTTTTTA	-	12-046,676
481	09772-L10187	*SPG11*	15q21	TTTCTTCAGGAT-TGATAGTCATTC	45.7 Mb to *IDH2*	15-042,706
276	17450-L29159	*GRIN2A*	16p13	TGCAGGATTATA-ATCTCACAATCT	-	16-009,761
255	22209-L31583	*POLR2A*	17p13	ACAACAAGAAGA-AGATCATCATCA	-	17-007,355
126	22212-L31462	*RNMT*	18p11	TACAATGAACTT-CAGGAAGTTGGT	-	18-013,724
208	16261-L18553	*SAMHD1*	20q11	AGTAGACAATGA-GTTGCGTATTTG	-	20-034,979

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

Related SALSA MLPA probemixes

ME012 MGMT-IDH1-IDH2	Contains probes for methylation detection of the *MGMT* gene and probes for detection of the most common *IDH1/2* mutations.
ME024 9p21 CDKN2A/2B region	Contains probes for both methylation and copy number detection of the 9p21.3 chromosomal region (*CDKN2A/B*, *MTAP*).
P027 Uveal melanoma	Contains several probes for the 1p chromosome arm, among others.
P105 Glioma-2	Contains probes for detection of copy number alterations of *PDGFRA*, *EGFR*, *CDKN2A*, *PTEN*, *CDK4*, *MIR26A2*, *MDM2*, *NFKB1A* and *TP53*.
P370 BRAF-IDH1-IDH2	Contains probes to detect genomic duplications leading to the *SRGAP3-RAF1*, *KIAA1549-BRAF* and *FGFR1-TACC1* fusion genes, to identify the most common *BRAF* and *IDH1/2* point mutations, and to detect copy number alterations of *BRAF*, *CDKN2A/B*, *FGFR1*, *MYB* and *MYBL1*.
P419 CDKN2A/2B-CDK4	Contains probes for *CDKN2A*, *CDKN2B* and *CDK4*.

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P088 product history	
Version	*Modification*
D1	Three target probes and four reference probes have been replaced, and an additional target probe for chromosome 19 has been added. Several probes have been changed in length with no change in sequence detected. Sample DNA used for this probemix has been changed from SD054 to SD079.
C2	One reference probe has been replaced and several probes have been changed in length with no change in sequence detected. Sample DNA used for this probemix has been changed from SD021 to SD054.
C1	Several target probes and all reference probes have been replaced. In addition, four probes for point mutations in *IDH1* and *IDH2*, and probes for *CDKN2A* and *CDKN2B* have been included.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	Several probes have been replaced, including three probes for 1q. In addition, extra control fragments have been included.
A1	First release.

Implemented changes in the product description
Version D1-02 – 22 March 2023 (04P) - Product description rewritten and adapted to a new template. - Intended purpose updated; copy number changes replaced by deletions, wording changed. - Clinical background updated to clarify the molecular definition of oligodendrogliomas according to the WHO. - Information about the minimal required tumour cell percentage and evaluation by a pathologist added to the required specimens section. - Positive control DNA samples section updated; chromosomal bands corrected for NA22976 and HT-1376, footnotes added to some of the tumour cells lines to indicate that also other probe targets are affected by copy number alterations. - Performance characteristics rephrased to be in line with the WHO definition of oligodendroglioma. - Information about interpretation of mutation-specific probes added to the P088 specific notes in the interpretation of results section. - Information added to the confirmation of results section indicating that presence of the *IDH1* p.R132H, *IDH1* p.R132C, *IDH2* p.R172K and *IDH2* p.R172M mutations can be confirmed by sequence analysis. - Exon numbering of the *CDKN2A* gene has been changed according to MANE transcripts. - NM_ references sequences for *CDKN2A* and *CDKN2B* have been changed according to MANE transcripts. - Ligation sites of the probes targeting the *CDKN2A* and *CDKN2B* genes added to Table 2. - Ligation sites of the probes targeting the *IDH1* gene updated according to new version of the NM_ reference sequence. - Warning about background signal for the IDH1 p.R132C mutation-specific probe (227 nt; 14787-L23353) added to Table 1 and 2. - Information on Related SALSA MLPA probemixes updated. - List of selected publications using SALSA MLPA Probemix P088 Oligodendroglioma 1p-19q updated. - P088 product history adjusted; modification in C1 version corrected. - UK has been added to the list of countries in Europe that accept the CE mark. Version D1-01 – 23 April 2020 (04) - P088-D1 is now CE marked. - NM sequence of *IDH2* gene updated to NM_002168.4. - Information on positive control DNA samples added on page 3. - Product description adapted to a new product version (version number changed, small changes in Table 1 and Table 2). - Product description rewritten and adapted to a new template. - A new SD is available for P088-D1. The sample DNA used for this probemix is changed from SD054 to SD079. - Related SALSA MLPA probemixes updated on page 9 (P027 and P419 added). - New selected publications for P088 added to page 10. - Gene name GPR98 changed to AVGRV1 according to HUGO nomenclature in Table 2b. Version C2-01 – 04 December 2018 (01P) - Product description restructured and adapted to a new template. - For uniformity, the chromosomal positions and bands in this document are now all based on hg18 (NCBI36). - Warning about SNPs removed in Table 1 and 2 for NOTCH2 (05745-L05183), CDKN2B (10337-L23606) and ZNF296 (03221-L24213) probes.

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