Product Description

SALSA® MLPA® Probemix P027-C2 Uveal melanoma

To be used with the MLPA General Protocol.

Version C2
For complete product history see page 10.

Catalogue numbers:

- **P027-025R:** SALSA MLPA Probemix P027 Uveal melanoma, 25 reactions.
- **P027-050R:** SALSA MLPA Probemix P027 Uveal melanoma, 50 reactions.
- **P027-100R:** SALSA MLPA Probemix P027 Uveal melanoma, 100 reactions.

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

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

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
The SALSA MLPA Probemix P027 Uveal melanoma is a **research use only (RUO)** assay for the detection of deletions or duplications in chromosomes 1p, 3, 6 and 8, which are recurrent genetic alterations in uveal melanoma.

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults, with mortality over 50%, usually due to metastatic spread to the liver. Research suggests that UMs with monosomy 3 (50-60% of all UMs) represent a distinct pathological entity as compared to those with normal disomy 3, as monosomy 3 strongly correlates with metastatic potential (Prescher et al. 1996). Chromosome 6 aberrations probably constitute a second entry point in the process of carcinogenesis, while gains in 8q seem to appear later in the natural history of UMs due to their higher frequency in larger tumours. Early detection of high risk UM patients would enable better screening for metastasis and for optimized therapy selection, and as well to reassure patients with no loss of chromosome 3, which is associated with good prognosis (for review see e.g. Jager et. 2020).

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.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *VHL, BAP1, FHIT* and *MYC* exon numbering used in this P027-C2 Uveal melanoma product description is the exon numbering from the LRG_322 (VHL), LRG_529 (BAP1), NG_007551.2 (FHIT), LRG_1397 (MYC) sequence. As changes to the databases can occur after release of this product description exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P027-C2 Uveal melanoma contains 50 MLPA probes with amplification products between 124 and 504 nucleotides (nt). This includes seven probes for 1p, 19 probes for chromosome 3, six probes chromosome 6 and six probes for chromosome 8. In addition, 12 reference probes are included that target relatively copy number stable regions in various cancer types including uveal melanoma. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

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

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

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

Required specimens
Extracted DNA, which includes DNA derived from paraffin-embedded tissues, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different healthy individuals without a history of uveal melanoma. 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. Sample ID numbers from the Coriell Institute listed in the table below have been tested with this P027-C2 probemix at MRC Holland and can be used as a positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position (hg18) of CNA*	Altered target genes in P027-C2	Expected copy number alteration
NA10985	Coriell Institute	3p25.3-p26.3	*CHL1, BRK1, VHL*	Heterozygous deletion
NA03503	Coriell Institute	3p25.1-p26.3	*CHL1, BRK1, VHL, PPARG, XPC*	Heterozygous duplication
NA04127	Coriell Institute	3p21.31-p26.3	*CHL1, BRK1, VHL, PPARG, XPC, MIR128-2, MLH1, CTNNB1, RBM5*	Heterozygous duplication
NA08778	Coriell Institute	3q11.2-q21.1	*PROS1, CASR*	Heterozygous deletion
NA03563	Coriell Institute	3q21.1-q29	*CASR, MME, OPA1*	Heterozygous duplication
NA10175	Coriell Institute	3q29	*OPA1*	Heterozygous duplication
NA22976	Coriell Institute	3q29	*OPA1*	Heterozygous duplication
NA22770	Coriell Institute	6p25.2	*ECI2*	Heterozygous duplication
NA09367	Coriell Institute	6q23.2	*CCN2*	Heterozygous duplication
NA06802	Coriell Institute	6q25.3	*IGF2R*	Heterozygous deletion
NA07994	Coriell Institute	6q25.3	*IGF2R*	Heterozygous duplication
NA03255	Coriell Institute	8p21.3	*LZTS1*	Heterozygous duplication
NA14485	Coriell Institute	8p12-p21.3	*LZTS1, NRG1*	Heterozygous duplication
NA02030	Coriell Institute	8p21.3-q24.21	*LZTS1, NRG1, RP1, MYC, ASAP1*	Heterozygous duplication
NA03999	Coriell Institute	8q24.21	*MYC, ASAP1*	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P027-C2 Uveal melanoma probemix.

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

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

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

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

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *NBL1* or *RUNX2* genes. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a 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.

P027 specific note

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

Limitations of the procedure

- In most populations, most genetic alterations in chromosomal regions 1p, 3, 6 and 8 are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P027 Uveal melanoma.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

COSMIC mutation database

http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

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

Table 1. SALSA MLPA Probemix P027-C2 Uveal melanoma

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	Chr 1	Chr 3	Chr 6	Chr 8
64-105	Control fragments – see table in probemix content section for more information					
124 ¥	Reference probe 21547-L02274	18q11				
130 ¥	Reference probe 18709-L24593	5q31				
136	**NOTCH2 probe** 02867-L02334		1p12			
141 Δ	**MLH1 probe** 15288-L20037			3p22.2		
148	Reference probe 04445-L03831	4q13				
154 ¥	**MYC probe** 20780-L27349					8**q**24.21
160 « ¥	**RUNX2 probe** 22020-L31270				6p12.3	
166	**MYC probe** 15894-L16789					8**q**24.21
172 *	Reference probe 09255-L31288	7q22				
178 ±	**FHIT probe** 02292-L02212			3p14.2		
184	**RP1 probe** 16641-L19172					8**q**12.1
190	**MIR128-2 probe** 15896-L17989			3p22.3		
196	**MFN2 probe** 04888-L04272		1p36.22			
202	**MME probe** 00487-L00069			3**q**25.31		
207	**CHL1 probe** 14147-L17987			3p26.3		
211 *	Reference probe 05281-L31289	14q22				
220	**GJB3 probe** 13671-L17892		1p34.3			
226	**BAP1 probe** 16643-L20039			3p21.1		
233	**IGF2R probe** 02798-L20038				6**q**25.3	
240 ¥	**RPE65 probe** 21549-L31285		1p31.3			
247	**CASR probe** 05708-L19175			3**q**21.1		
255 *	Reference probe 13573-L31290	19p13				
260	**PPARG probe** 06900-L06480			3p25.2		
266 +	**CCN2 probe** 04745-L19276				6**q**23.2	
275 « ¥	**NBL1 probe** 04148-L31286		1p36.13			
283	**BRK1 probe** 15895-L18089			3p25.3		
292	Reference probe 08936-L09031	11p15				
299	**ASAP1 probe** 16241-L18499					8**q**24.21
310	**LZTS1 probe** 02552-L19178					8p21.3
318	**NRG1 probe** 04239-L03575					8p12
328	**PROS1 probe** 05297-L04685			3**q**11.2		
337	**FHIT probe** 02290-L01781			3p14.2		
346	**OPA1 probe** 03271-L02708			3**q**29		
355	**PTAFR probe** 02267-L01425		1p35.3			
364	**RBM5 probe** 15897-L18094			3p21.31		
373	Reference probe 09779-L10194	15q15				
383	**ECI2 probe** 10252-L11363				6p25.2	
391	**VHL probe** 13322-L14735			3p25.3		
400	Reference probe 14423-L16128	12q21				
409 ¥	**ROBO1 probe** 21548-L31318			3p12.3		
418	**MUTYH probe** 03964-L03351		1p34.1			
427	**CDKN1A probe** 00585-L18090				6p21.31	
436	Reference probe 12790-L19277	2q13				
445	**VHL probe** 15899-L18091			3p25.3		
454	**CTNNB1 probe** 16407-L18832			3p22.1		
463	**DCDC2 probe** 10253-L18092				6p22.2	
474	**XPC probe** 06118-L05573			3p25.1		
483	**BAP1 probe** 16644-L19176			3p21.1		
490	Reference probe 14909-L17745	18p11				
504 ¥	Reference probe 09870-L19465	2p15				

[a] See section Exon numbering on page 1 for more information.

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.
± SNP rs137953369 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.
+ *CTGF* gene name has been updated to *CCN2* according to the latest HGNC nomenclature.

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

Table 2. P027-C2 Uveal melanoma probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene (exon[a])	Location (hg18)	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Loss of 1p arm Studies have shown a correlation between loss of the 1p arm and poor prognosis in UM, in particular for patients with loss of both the 1p arm and of chromosome 3 (Kilic et al. 2005).					
196	04888-L04272	*MFN2*	1p36.22	CTGGTGGACGAT-TACCAGATGGAC	7.9 **M**b
275 «	04148-L31286	*NBL1*	1p36.13	AGCTGCACAATT-TAATATATTCAA	8.5 **M**b
355	02267-L01425	*PTAFR*	1p35.3	CATCTTCATCGT-GTTCAGCTTCTT	6.7 **M**b
220	13671-L17892	*GJB3*	1p34.3	CCATGGGAGTGT-GTCAGGTGGAAG	10.6 **M**b
418	03964-L03351	*MUTYH*	1p34.1	CTCATACCATCT-ATTCAGAGACGT	23.1 **M**b
240	21549-L31285	*RPE65*	1p31.3	ATGCCCTTGTTA-ATGTCTACCCAG	51.6 **M**b
136	02867-L02334	*NOTCH2*	1p12	AAGCTGCAGACA-TCCGTAGGACAC	-
Loss of chromosome 3 Monosomy of chromosome 3 is the most common chromosomal abnormality detected in ~50% of UM patients, and is strongly associated with short survival (Prescher et al. 1996). Several different candidate genes have been reported, including *BAP1* (Harbour et al. 2010), *RBM5, PPARG, ROBO1* (Lake et al. 2010) as well as the tumour suppressor genes *VHL* at 3p25.3, and *FHIT* at 3p14.2.					
207	14147-L17987	*CHL1*	3p26.3	CCTAGGTGCTGT-AAACTGCAAACC	9.9 **M**b
283	15895-L18089	*BRK1*	3p25.3	AAAAGGTGAGAC-ACTCACCTAGAA	20.3 kb
391	13322-L14735	*VHL*, ex 2	3p25.3	CGTCAACATTGA-GAGATGGCACAA	3.3 kb
445	15899-L18091	*VHL*, ex 3	3p25.3	CCAAATGTGCAG-AAAGACCTGGAG	2.2 **M**b
260	06900-L06480	*PPARG*	3p25.2	ATACAACAAGGC-CATTTTCTCAAA	1.8 **M**b
474	06118-L05573	*XPC*	3p25.1	AGCAAGAGTGGT-GAGGCTTGGAGA	21.6 **M**b
190	15896-L17989	*MIR128-2*	3p22.3	GAGAGTGAGTAG-CAGGTCTCACAG	1.2 **M**b
141 Δ	15288-L20037	*MLH1*	3p22.2	TCTAACGCGCAA-GCGCATATCCTT	4.2 **M**b
454	16407-L18832	*CTNNB1*	3p22.1	GCTGACTATCCA-GTTGATGGGCTG	8.8 **M**b
364	15897-L18094	*RBM5*	3p21.31	ATATGATGACTA-CCGAGACTATGA	2.3 **M**b
483	16644-L19176	*BAP1*, ex 9	3p21.1	AACCTGATGGCA-GTGGTGCCCGAC	2.2 kb
226	16643-L20039	*BAP1*, ex 4	3p21.1	ATACGTCCGTGA-TTGATGATGATA	8.1 **M**b
178 ±	02292-L02212	*FHIT*, ex 5	3p14.2	GAGGACATGTCG-TTCAGATTTGGC	285.0 kb
337	02290-L01781	*FHIT*, ex 4	3p14.2	CCTGCCTGCTTA-GACCCTCTATAA	18.0 **M**b
409	21548-L31318	*ROBO1*	3p12.3	ATATGGATACGG-ATGCGCCAGAAG	16.4 **M**b
328 #	05297-L04685	*PROS1*	3**q**11.2	TGTGAATGCCCC-GAAGGCTACAGA	28.4 **M**b
247	05708-L19175	*CASR*	3**q**21.1	CTCCATCGTGTT-TAAGGAAGTCGG	32.9 **M**b
202	00487-L00069	*MME*	3**q**25.31	CGTTGACTGGTG-GACTCAACAGTC	38.5 **M**b
346	03271-L02708	*OPA1*	3**q**29	TGAAGATGGTGA-GAAGAAGATTAA	-
Gain of 6p and loss of 6q Gain of 6p and loss of 6q, often due to isochromosome 6p, are associated with good prognosis in UM (White et al. 1998).					
383	10252-L11363	*ECI2*	6p25.2	AAGGACTTGTTA-CTGAAGTTTTCC	20.3 **M**b
463	10253-L18092	*DCDC2*	6p22.2	GCAGAGAGGTCT-GAAACACGGGGG	12.4 **M**b
427	00585-L18090	*CDKN1A*	6p21.31	CGGCTGATCTTC-TCCAAGAGGAAG	8.7 **M**b
160 «	22020-L31270	*RUNX2*	6p12.3	GTTGTGATGCGT-ATTCCCGTAGAT	86.8 **M**b

266 +	04745-L19276	*CCN2*	6**q**23.2	ACCGAGCTAAAT-TCTGTGGAGTAT	28.0 **M**b
233	02798-L20038	*IGF2R*	6**q**25.3	TTCAACACAACA-GTGAGCTGTGAC	-
Loss of 8p and gain of 8q 8p loss and 8q gain, often due to isochromosome 8q, are associated with poor prognosis in UM. Several potential target genes have been suggested, such as *LZTS1* at 8p21.3 (Onken et al. 2008), *MYC* at 8q24.21, and *ASAP1* at 8q24.21 (Ehlers et al. 2005).					
310	02552-L19178	*LZTS1*	8p21.3	GCTGCAGCGCAA-GAAGAACGAGGC	12.6 **M**b
318	04239-L03575	*NRG1*	8p12	CTGGGACAAGCC-ATCTTGTAAAAT	23.0 **M**b
184	16641-L19172	*RP1*	8**q**12.1	ATCCTGAGCTCT-GGAGCTGTGGTG	73.1 **M**b
166	15894-L16789	*MYC*, ex 1	8**q**24.21	CTGGAACTTACA-ACACCCGAGCAA	4.3 kb
154	20780-L27349	*MYC*, ex 3	8**q**24.21	GAACGAGCTAAA-ACGGAGCTTTTT	2.3 **M**b
299	16241-L18499	*ASAP1*	8**q**24.21	TTCCTTTCAGGC-TGTCCTTCGATG	-

[a] See section Exon numbering on page 1 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.

± SNP rs137953369 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
This probe'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.
+ *CTGF* gene name has been updated to *CCN2* according to the latest HGNC nomenclature.

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

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
504	09870-L19465	*PEX13*	2p15	TGAGGATGACCA-TGTAGTTGCCAG	02-061,126
436	12790-L19277	*EDAR*	2q13	AGAATCAAGGCT-TTTGTGATATGT	02-108,880
148	04445-L03831	*GNRHR*	4q13	GGACTGGTCTAA-GCTGCTCAAGAT	04-068,304
130	18709-L24593	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132,038
172	09255-L31288	*SLC26A4*	7q22	CTGGGGCTGGAT-CTCGGTTTACTA	07-107,124
292	08936-L09031	*SLC6A5*	11p15	TGTTTGCCTCCT-TTGTGTCTGTAC	11-020,586
400	14423-L16128	*ALX1*	12q21	ATGACACCTTAT-TCTCACTCGCCT	12-084,219
211	05281-L31289	*ATL1*	14q22	GTGGCTGAATCT-CTCAAAGTTGAC	14-050,128
373	09779-L10194	*SPG11*	15q15	CCAGTGTAAGCA-GTATGCTATTGG	15-042,690
490	14909-L17745	*RNMT*	18p11	TACAATGAACTT-CAGGAAGTTGGT	18-013,724
124	21547-L02274	*NPC1*	18q11	GACGAGTCTGTG-GATGAGGTCACA	18-019,394
255	13573-L31290	*CACNA1A*	19p13	CTCGGCTTAGGC-AAGAAATGTCCT	19-013,201

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

Related SALSA MLPA probemixes

P016 VHL	Contains at least two probes for each exon of *VHL* and several flanking probes on 3p25-p26
P175 Tumour gain	Contains two probes for *MYC* gene on 8q24.21
P417 BAP1	Contains probes for each exon of *BAP1* gene on 3p21.1

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.
- Ehlers JP et al. (2005) DDEF1 is located in an amplified region of chromosome 8q and is overexpressed in uveal melanoma. *Clin Cancer Res*. 11:3609-13.
- Harbour JW et al. (2010) Frequent mutation of BAP1 in metastasizing uveal melanomas. Science. 330:1410-3.
- Hömig-Hölzel C and Savola S. (2012) Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Jager MJ et al. (2020) Uveal melanoma. *Nat Rev Dis Primers*. 6(1):24.
- Kilic E et al. (2005) Concurrent loss of chromosome arm 1p and chromosome 3 predicts a decreased disease-free survival in uveal melanoma patients. *Invest Ophthalmol Vis Sci*. 46:2253-7.
- Lake SL et al. (2010) Whole-genome microarray detects deletions and loss of heterozygosity of chromosome 3 occurring exclusively in metastasizing uveal melanoma. *Invest Ophthalmol Vis Sci.* 51:4884- 91.Onken M et al. (2008) A metastasis modifier locus on human chromosome 8p in uveal melanoma identified by integrative genomic analysis. *Clin Cancer Res.* 14:3737-45.
- Prescher G et al. (1996) Prognostic implications of monosomy 3 in uveal melanoma. *Lancet.* 347:1222–5
- 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.
- 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.
- White VA et al. (1998) Correlation of cytogenetic abnormalities with the outcome of patients with uveal melanoma. *Cancer*. 83:354-9.

Selected publications using SALSA MLPA Probemix P027 Uveal melanoma

- Beasley AB et al. (2020) Low-Pass Whole-Genome Sequencing as a Method of Determining Copy Number Variations in Uveal Melanoma Tissue Samples. *J Mol Diagn.* 22:429-34.
- Damato B et al. (2009) Multiplex ligation-dependent probe amplification of uveal melanoma: correlation with metastatic death. *Invest Ophthalmol Vis Sci.* 50:3048-55.
- Damato BE et al. (2010) Genotypic profiling of 452 choroidal melanomas with Multiplex Ligation-Dependent Probe Amplification. *Clin Cancer Res*. 16:6083-92.
- Dogrusoz M et al. (2017) The prognostic value of AJCC staging in uveal melanoma is enhanced by adding chromosome 3 and 8q status. *Invest Ophthalmol Vis Sci*. 58:833-42.
- Dopierala J et al. (2011) Genetic heterogeneity in uveal melanoma assessed by multiplex ligation-dependent probe amplification. *Invest Ophthalmol Vis Sci*. 51:4898-905.
- Lake SL et al. (2010) Whole-genome microarray detects deletions and loss of heterozygosity of chromosome 3 occurring exclusively in metastasizing uveal melanoma. *Invest Ophthalmol Vis Sci*. 51:4884-91.
- Lake SL et al. (2011) Multiplex ligation-dependent probe amplification analysis of uveal melanoma with extraocular extension demonstrates heterogeneity of gross chromosomal abnormalities. *Invest Ophthalmol Vis Sci*. 52:5559-64.
- Lake SL et al. (2011) Multiplex ligation-dependent probe amplification of conjunctival melanoma reveals common BRAF V600E gene mutation and gene copy number changes. *Invest Ophthalmol Vis Sci.* 52:5598-604.

- Lake SL et al. (2012) Comparison of formalin-fixed and snap-frozen samples analyzed by multiplex ligation-dependent probe amplification for prognostic testing in uveal melanoma. *Invest Ophthalmol Vis Sci.* 53:2647-52.
- Larsen AC et al. (2014) MicroRNA expression analysis and Multiplex ligation-dependent probe amplification in metastatic and non-metastatic uveal melanoma. *Acta Ophthalmol*. 92:541-9.
- Narasimhaiah D et al. (2019) DNA alteration-based classification of uveal melanoma gives better prognostic stratification than immune infiltration, which has a neutral effect in high-risk group. *Cancer Med.* 8:3036-46.
- Patrone S et al. (2018) Prognostic value of chromosomal imbalances, gene mutations, and BAP1 expression in uveal melanoma. *Genes Chromosomes Cancer*. 57:387-400.
- Shain AH et al. (2019) The genetic evolution of metastatic uveal melanoma. *Nat Genet*. 51:1123-30.
- Vaarwater J et al. (2012) Multiplex ligation-dependent probe amplification equals fluorescence in-situ hybridization for the identification of patients at risk for metastatic disease in uveal melanoma. *Melanoma Res*. 22:30-7.

P027 product history	
Version	*Modification*
C2	Three reference probes have been replaced and the lengths of several probes have been adjusted.
C1	Contents completely revised: several target probes have been added or replaced and several reference probes have been replaced. New D-control fragments (88 and 96 nt) have been included (QDX2).
B1	Several probes have been replaced by other probes in the same chromosomal region as compared to the previous version.
A1	First release.

Implemented changes in the product description

Version C2-03 – 09 March 2021 (04P)
- Product description rewritten and adapted to a new template.
- Positive samples for P027 probemix listed on page 3.
- *CTGF* gene name has been updated to *CCN2* according to the latest HGNC nomenclature.
- Warning added to Tables 1 and 2 about variability of MLH1 probe (15288-L20037) at 141 nt.
- Warning added to Tables 1 and 2 about SNP affecting signal of FHIT probe (02292-L02212) at 178 nt.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- New reference added to Selected publications using P027 on page 9 and 10.

Version C2-02 – 09 November 2018 (01P)
- Implemented changes of product description version C2-01 corrected.
- Several minor textual and layout changes.

Version C2-01 – 28 September 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Various minor textual or layout changes.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene; other warnings removed.
- Probe locations in Table 1 and 2 are now consistently given according to hg18.

Version 15 (T06)
- Product description adapted to a new lot (lot number added, new picture included).
- New references added on page one.

- Warning added in Table 1 and 2 for probes at 160 nt, 184 nt, 318 nt, 345 nt, 474 nt.
- C3ORF10 gene has been renamed to BRK1 in Table 1 and 2.
- Various textual changes.

Version 14 (49)
- Product description adapted to a new lot (lot number added, new picture included).
- New references added on page one.
- Warning added in Table 1 and 2 for probes at 178 nt, 241 nt and 328 nt.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200