Product Description
SALSA® MLPA® Probemix P258-C2 SMARCB1

To be used with the MLPA General Protocol.

Version C2
For complete product history see page 10.

Catalogue numbers:
- **P258-025R:** SALSA MLPA Probemix P258 SMARCB1, 25 reactions.
- **P258-050R:** SALSA MLPA Probemix P258 SMARCB1, 50 reactions.
- **P258-100R:** SALSA MLPA Probemix P258 SMARCB1, 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 P258 SMARCB1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SMARCB1* gene, inactivation of which has been associated with malignant rhabdoid tumours (MRTs). Rhabdoid tumours are a highly malignant group of neoplasms that usually occur in children under two years of age. MRTs of the kidney were first identified as a sarcomatous variant of Wilms tumours (Beckwith and Palmer, 1978). Later, extrarenal rhabdoid tumours were reported in numerous locations, including the central nervous system (CNS) (Parham et al., 1994). Classification has been difficult because of considerable variation in the histologic and immunologic characteristics within and between rhabdoid tumours of the liver, soft tissues, and CNS. In the CNS, rhabdoid tumours may be pure rhabdoid tumours or a variant that has been designated atypical teratoid/rhabdoid tumour (AT/RT) (Zin et al., 2021).

Germline alterations in *SMARCB1* have also been associated with several genetic syndromes, including Coffin-Siris Syndrome 3 and *SMARCB1*-related schwannomatosis (Plotkin et al., 2022). Coffin-Siris Syndrome 3 (CSS3) is a congenital malformation syndrome characterized by developmental delay and intellectual disability, among other variable features. Patients with *SMARCB1* alterations may have more severe neurodevelopmental deficits and structural brain abnormalities (Kosho et al., 2014). *SMARCB1*-related schwannomatosis (SWN) is characterized by the onset of multiple intracranial, spinal, or peripheral schwannomas. Affected individuals may also present multiple meningiomas. Individual schwannoma tumours from patients with schwannomatosis have been found to harbour somatic mutations in *SMARCB1* (Sestini et al., 2008).

The protein encoded by the *SMARCB1* gene (also known as INI1 or SNF5) is a core component of the SWI/SNF complex which is actively involved in remodelling chromatin structures, allowing the transcriptional machinery to access its targets more effectively. This ATP-dependent chromatin-remodelling complex plays important roles in cell proliferation and differentiation, in cellular antiviral activities and inhibition of tumour formation (Kalimuthu et al., 2016).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK469816/

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
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/

Exon numbering
The *SMARCB1* exon numbering used in this P258-C2 SMARCB1 product description is the exon numbering from the RefSeq transcript NM_003073.5. The exon numbering and NM_ sequence have been retrieved on 07/2023 and we have adopted MANE Select exon numbering. 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 P258-C2 SMARCB1 contains 42 MLPA probes with amplification products between 127 and 445 nucleotides (nt). This includes two probes for each exon of the *SMARCB1* gene (nine exons). In addition, this probemix includes 10 probes flanking *SMARCB1* in 22q11 and 22q12 chromosomal regions, and 14 reference probes that detect several autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 2b 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 reference 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 healthy individuals without a history of rhabdoid tumours and other *SMARCB1*-associated syndromes and cancers. 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 NA02325 and NA07106 from the Coriell Institute have been tested with this P258-C2 probemix at MRC Holland and can be used as a positive control samples to detect heterozygous duplications in the *SMARCB1* gene and in the surrounding genomic regions targeted by flanking probes. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Expected copy number alteration (CNA)*
NA02325	Coriell Institute	Heterozygous duplication of 22q11.21-q12.2 including ***SMARCB1*** and flanking genes *TBX1, DGCR8, SNAP29, LZTR1, PPIL2, GNAZ, SNRPD3, SEZ6L* and *NIPSNAP1*.
NA07106	Coriell Institute	Heterozygous duplication of 22q11.21-q12.2 including ***SMARCB1*** and flanking genes *TBX1, DGCR8, SNAP29, LZTR1, PPIL2, GNAZ, SNRPD3, SEZ6L* and *NIPSNAP1*.
KP-363T ◊ (ACC 808)	DSMZ	Heterozygous deletion of 22q11.21-q12.2 including ***SMARCB1*** and flanking genes *TBX1, DGCR8, SNAP29, LZTR1, PPIL2, GNAZ, SNRPD3, SEZ6L* and *NIPSNAP1*.

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in these samples cannot be determined by this P258-C2 SMARCB1 probemix.
◊ CNAs detected by reference probes are not reported for this sample.

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 reference probe over all the reference samples should be ≤0.10. When these criterion are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders

as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. 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.

P258-C2 SMARCB1 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 *SMARCB1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P258 SMARCB1.
- 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 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 duplication of *SMARCB1* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P258-C2 SMARCB1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			Location (hg18) in kb
		Reference	***SMARCB1***	Flanking	
64-105	Control fragments – see table in probemix content section for more information				
127 ¥	Reference probe 18709-L26552	5q31			05-132,038
137	Reference probe 03797-L04594	21q22			21-037,920
144 ¬	GNAZ probe 08477-L08488			22q11.22	22-021,768
150 ¬	DGCR8 probe 08475-L20825			22q11.21	22-018,454
155	Reference probe 08375-L20826	15q24			15-072,881
160	**SMARCB1 probe** 08283-L20827		**Exon 3**		22-022,466
166 ¬	SNRPD3 probe 08481-L20828			22q11.23	22-023,284
172	**SMARCB1 probe** 08295-L08109		**Exon 9**		22-022,506
177 ± « ¬	TBX1 probe 05408-L19742			22q11.21	22-018,127
184	**SMARCB1 probe** 08292-L20678		**Exon 7**		22-022,498
190	**SMARCB1 probe** 08287-L08101		**Exon 5**		22-022,475
196	**SMARCB1 probe** 16893-L19747		**Exon 2**		22-022,464
202	Reference probe 05706-L20677	3q21			03-123,459
208	**SMARCB1 probe** 08288-L08102		**Exon 5**		22-022,476
214	**SMARCB1 probe** 08282-L08096		**Exon 2**		22-022,464
220	Reference probe 08879-L08935	2p23			02-025,237
230 ¥	**SMARCB1 probe** 18230-L23153		**Exon 6**		22-022,489
238 ¥	Reference probe 14498-L23152	20p12			20-010,578
245	**SMARCB1 probe** 08296-L19752		**Exon 9**		22-022,507
251 « ¬	TBX1 probe 10810-L19753			22q11.21	22-018,133
256	**SMARCB1 probe** 08284-L08098		**Exon 3**		22-022,466
265	**SMARCB1 probe** 08286-L08100		**Exon 4**		22-022,473
275 ¬	SEZ6L probe 05929-L05810			22q12.1	22-025,018
283	**SMARCB1 probe** 08289-L08103		**Exon 6**		22-022,489
292	**SMARCB1 probe** 08293-L08107		**Exon 8**		22-022,506
301	Reference probe 12783-L13918	2q13			02-108,889
310	**SMARCB1 probe** 08280-L20836		**Exon 1**		22-022,459
319	**SMARCB1 probe** 08294-L20837		**Exon 8**		22-022,506
328	Reference probe 04007-L03430	2q33			02-203,087
336	**SMARCB1 probe** 08279-L08093		**Exon 1**		22-022,459
346	Reference probe 06560-L06118	1q32			01-199,601
355	**SMARCB1 probe** 08285-L19741		**Exon 4**		22-022,473
364	**SMARCB1 probe** 08291-L08105		**Exon 7**		22-022,497
373 ¬	SNAP29 probe 16748-L19368			22q11.21	22-019,565
382 *	Reference probe 01522-L00952	10p14			10-011,248
391 ¬	NIPSNAP1 probe 02580-L02042			22q12.2	22-028,282
400	Reference probe 10670-L11252	6p12			06-052,033
409 ¬	PPIL2 probe 05467-L20675			22q11.21	22-020,380
418 ¬	LZTR1 probe 01521-L00951			22q11.21	22-019,679
427	Reference probe 06942-L06522	11q12			11-061,486
436	Reference probe 09077-L09246	19p13			19-013,207
445	Reference probe 08793-L08817	10q21			10-055,261

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

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.
± SNV rs72646950 could influence the probe signal at 177nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P258-C2 probes arranged according to chromosomal location

Table 2a. **SMARCB1** and flanking probes

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
Flanking probes centromeric to *SMARCB1* gene						
177 ± « ¬	05408-L19742	*TBX1*	22q11.21	CCGGGTGAAGCT-TCGCTGGCTGCC	6,2 kb	22-018,127
251 « ¬	10810-L19753	*TBX1*	22q11.21	TCCCTTCGCGAA-AGGCTTCCGGGA	320,3 kb	22-018,133
150 ¬	08475-L20825	*DGCR8*	22q11.21	GGTAATGGACGT-TGGCTCTGGTGG	1,1 **M**b	22-018,454
373 ¬	16748-L19368	*SNAP29*	22q11.21	GTATCCACTTAC-CTGTATCATCCA	113,8 kb	22-019,565
418 ¬	01521-L00951	*LZTR1*	22q11.21	ATGATGAAGGAG-TTCGAGCGCCTC	700,5 kb	22-019,679
409 ¬	05467-L20675	*PPIL2*	22q11.21	GAAGAGCCCTCA-ACCAGTGCCACT	1,4 **M**b	22-020,380
144 ¬	08477-L08488	*GNAZ*	22q11.22	TGACCGCCACCT-GCGCTCAGAGAG	690,9 kb	22-021,768
SMARCB1 gene at 22q11.23. Ligation sites are indicated according to NM_003073.5						
		start codon	*205-207 (exon 1)*			
336	08279-L08093	upstream	259 nt before exon 1	ACCACCCAGGCT-TCCAGATACTAG	0,5 kb	22-022,459
310	08280-L20836	exon 1	226-227	TGGCGCTGAGCA-AGACCTTCGGGC	4,7 kb	22-022,459
196	16893-L19747	exon 2	25 nt after exon 2	AGGGTTTGTAAA-CCTGTTTCAAAA	0,2 kb	22-022,464
214	08282-L08096	exon 2	277 nt after exon 2, reverse	TCCTAAAACGTT-TTGAGACCAACA	1,4 kb	22-022,464
160	08283-L20827	exon 3	455-456	ATACACGACTCT-AGCCACCAGTGT	0,1 kb	22-022,466
256	08284-L08098	exon 3	531-530, reverse	CTGATGGACACA-GCCTTGTACTTC	7,4 kb	22-022,466
355	08285-L19741	exon 4	632-633	CCACCACTTAGA-TGCCGTGCCATG	0,1 kb	22-022,473
265	08286-L08100	exon 4	44 nt after exon 4, reverse	GCTGGAGAACTA-AGGCGGAATCAG	2,2 kb	22-022,473
190	08287-L08101	exon 5	730-731	ACCCAGCTGTGA-TCCATGAGAACG	0,1 kb	22-022,475
208	08288-L08102	exon 5	814-815	TGCGAGACGCCT-TCACCTGGAACA	13,4 kb	22-022,476
283	08289-L08103	exon 6	856-857	CGCCTGAGATGT-TTTCAGAAATCC	0,1 kb	22-022,489
230 ¥	18230-L23153	exon 6	997-998	GCGTCATCATCA-AGGTAGGTGACT	8,3 kb	22-022,489
364	08291-L08105	exon 7	1018-1019	TCCATGTGGGAA-ACATTTCCCTGG	0,1 kb	22-022,497
184	08292-L20678	exon 7	1147-1148	TCGCATACAGCA-TCCGGGGACAGC	8,2 kb	22-022,498
292	08293-L08107	exon 8	1196-1195, reverse	TGGGCAGAGGGT-TCTCGCTACGAG	0,1 kb	22-022,506
319	08294-L20837	exon 8	1291-1292	CTGAGATGGAGA-AGAAGATCCGCG	0,6 kb	22-022,506
172	08295-L08109	exon 9	1453-1452, reverse	TGGCGCTGGGCT-GTCCCCTCGCCT	0,3 kb	22-022,506
245	08296-L19752	exon 9	1793-1794	GGTATGTGAACA-AGGTTGGCACAC	776,9 kb	22-022,507
		stop codon	*1360-1362 (exon 9)*			
Flanking probes telomeric to *SMARCB1* gene						
166 ¬	08481-L20828	*SNRPD3*	22q11.23	CCGGTGAGGTAT-ATCGGGGGAAGC	1,7 **M**b	22-023,284
275 ¬	05929-L05810	*SEZ6L*	22q12.1	ACAGTCGGCGGA-AGTGCTGGGCGA	3,2 **M**b	22-025,018
391 ¬	02580-L02042	*NIPSNAP1*	22q12.2	AGGCTGACAAGT-TCTGAGGATTAC	-	22-028,282

[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.

¥ Changed in version C2. Minor alteration, no change in sequence detected.
± SNV rs72646950 could influence the probe signal at 177nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2b. Reference probes

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
346	06560-L06118	*TNNT2*	1q32	TTGAGAGAAACG-AGCTCCTCCTCC	01-199,601
220	08879-L08935	*POMC*	2p23	TCAGCCTCTTAA-AGCTGCCTGTAG	02-025,237
301	12783-L13918	*EDAR*	2q13	CTCCACACACGT-TGGCATACACAT	02-108,889
328	04007-L03430	*BMPR2*	2q33	AGATGAGACAAT-AATCATTGCTTT	02-203,087
202	05706-L20677	*CASR*	3q21	GATACAGGATAT-TTGACACTTGCA	03-123,459
127 ¥	18709-L26552	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132,038
400	10670-L11252	*PKHD1*	6p12	TTCAGTTGGTCA-GAGGAACCAAGG	06-052,033
382 *	01522-L00952	*CELF2*	10p14	TCCCCCGGTCAT-GGTCGGAAAAGG	10-011,248
445	08793-L08817	*PCDH15*	10q21	AGGAGGACGCAT-TCTGGAGATCCG	10-055,261
427	06942-L06522	*BEST1*	11q12	GACGAGGAGGAT-GCTCACGCTGGC	11-061,486
155	08375-L20826	*CSK*	15q24	TTTCGGAATCCT-TCTCTGGGAAAT	15-072,881
436 #	09077-L09246	*CACNA1A*	19p13	GATCGCCATGCT-CTTCTTCATCTA	19-013,207
238 ¥	14498-L23152	*JAG1*	20p12	AGGAGGCGTCAT-TCTGACACTGGC	20-010,578
137	03797-L04594	*KCNJ6*	21q22	CTCGAAGCTCCT-ACATCACCAGTG	21-037,920

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

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Related SALSA MLPA probemixes

P250 DiGeorge	Contains more probes in the 22q11 region.
P324 22q11	Contains more probes in the 22q11 region.
P294 Tumour Loss	Contains two probes for *SMARCB1*, among other genes.
P455 LZTR1	Contains probes for *LZTR1* gene. Alterations in *LZTR1* are associated with schwannomatosis.

References

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P258 product history	
Version	*Modification*
C2	One reference probe replaced.
C1	One target probe, several reference probes and one flanking probe replaced. Two TBX1 probes added. 88 and 96nt control fragments replaced (QDX2).
B1	Three reference probes and two flanking probes replaced.
A1	First lot of this product.

Implemented changes in the product description

Version C2-02 — 19 July 2023 (04P)
- Product description rewritten and adapted to a new template.
- General information and new references added on pages 9 and 10.
- Updated RefSeq transcript from NM_003073.3 to NM_003073.5.
- Links to Gene structure and transcript variants updated.
- Positive control sample KP-363T (ACC 808) from DSMZ added to the Positive control DNA samples section on page 3.
- Warning added for the 436 nt probe in Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- SNV warning added for the 177 nt probe in Table 1 and Table 2.
- New related SALSA MLPA probemix P455 LZTR1 added.
- Various minor textual and table layout changes.

Version C2-01 — 30 August 2019 (02P)
- Product description rewritten and adapted to a new template.
- Various minor textual and layout changes.
- Ligation sites of the probes targeting the SMARCB1 gene updated according to LRG_520.
- Positive cell line sample information included on page 2.
- Table 2b (reference probes arranged according to chromosomal location) added.
- New references added on pages 9-10.

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