Product Description SALSA® MLPA® Probemix P158-D1 JPS

To be used with the MLPA General Protocol.

Version D1. As compared to version C2, one *BMPR1A* and *SMAD4* probe replaced, one *PTEN* probe added, one *SMAD4* flanking probe removed, two reference probes replaced and one added. Also, the length of several probes has been adjusted. For complete product history see page 7.

Catalogue numbers:
- **P158-025R:** SALSA MLPA Probemix P158 JPS, 25 reactions.
- **P158-050R:** SALSA MLPA Probemix P158 JPS, 50 reactions.
- **P158-100R:** SALSA MLPA Probemix P158 JPS, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

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

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

General Information: The SALSA MLPA Probemix P158 JPS is a **research use only (RUO)** assay for the detection of deletions or duplications in the *BMPR1A*, *SMAD4*, and *PTEN* genes, which are associated with Juvenile Polyposis Syndrome (JPS).

JPS is an autosomal dominant condition characterised by the development of various types of tumours. The diagnosis is based on the occurrence of hamartomatous gastrointestinal polyps that turn into malignant lesions in approximately 20% of the cases. Defects in the *BMPR1A* gene on chromosome 10q23.2 and the *SMAD4* gene on chromosome 18q21.2 are the main causes of juvenile polyposis. Defects in the *PTEN* gene on chromosome 10q23.31 can result in Cowden syndrome which has some phenotypic resemblance to JPS. For this reason, probes for the *PTEN* gene are also included. More *PTEN* probes are present in the P225 PTEN probemix. Due to the presence of pseudogenes in the human genome, it was necessary to locate several probes in intron sequences close to the exons.

A study revealed a *SMAD4* retrotransposon in a breast cancer case using NGS data and multiple analytical methods (Patil et al. 2015). Another recent investigation on *SMAD4* supports the presence of a pseudogene, which has the potential to confound the interpretation of genetic testing results, as only mutations in the native gene are clinically significant (Mancini et al. 2015; Millson et al. 2015).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1469/.

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/

Probemix content: The SALSA MLPA Probemix P158-D1 JPS contains 51 MLPA probes with amplification products between 118 and 499 nt. This includes 15 probes for all 13 exons of the *BMPR1A* gene, with two probes for exon 1 and 3, and 15 probes for all 12 exons of the *SMAD4* gene, including four probes for exon

1. Furthermore, 11 probes for all nine exons of the *PTEN* gene are included, with two probes for exon 3 and one probe upstream of the gene. In addition, ten reference probes are included and detect different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

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

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

No DNA controls results in only five major peaks shorter than 105 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 105 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

Required specimens: Extracted DNA 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.

Reference samples: 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 unrelated individuals who are from families without a history of JPS. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change, therefore samples should be validated before use.

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

Interpretation of results: The standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

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

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *BMPR1A*, *SMAD4*, and *PTEN* gene are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemix P158 JPS.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

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.

***SMAD4*, *BMPR1A*, and *PTEN* mutation database:** http://www.arup.utah.edu/database/SMAD4/SMAD4_welcome.php, https://databases.lovd.nl/shared/genes/BMPR1A and https://databases.lovd.nl/shared/genes/PTEN. We strongly encourage users to deposit positive results in the University of Utah *SMAD4* database and the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *BMPR1A* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P158-D1 JPS

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)			
		Reference	*SMAD4*	*BMPR1A*	*PTEN*
64-105	Control fragments – see table in probemix content section for more information				
118 *	Reference probe 19041-L24884	5q32			
124 «	**SMAD4 probe** S0151-L14963		**Exon 1**		
130	Reference probe 00797-L00463	5q31			
137 «	**BMPR1A probe** 07189-L15131			**Exon 1**	
142	**SMAD4 probe** 02127-L01638		**Exon 2**		
148	**PTEN probe** 19343-L25668				**Exon 7**
154	Reference probe 03931-L03386	15q21			
160	**BMPR1A probe** 07197-L06812			**Exon 10**	
166 «	**SMAD4 probe** 07796-L08332		**Exon 1**		
172	**BMPR1A probe** 16647-L19180			**Exon 8**	
178	**PTEN probe** 16648-L19181				**Exon 3**
184	**PTEN probe** 11280-L15522				**Promoter**
190	**PTEN probe** 06729-L06339				**Exon 2**
196	**SMAD4 probe** 05147-L07333		**Exon 8**		
203	Reference probe 04732-L04149	7q21			
211	**BMPR1A probe** 16649-L19182			**Exon 11**	
218 ¥	**BMPR1A probe** 21424-L29928			**Exon 2**	
222 ¥ «	**BMPR1A probe** 11840-L31344			**Exon 1**	
229	**PTEN probe** 03718-L02944				**Exon 4**
238 *	**SMAD4 probe** 21420-L30664		**Exon 7**		
244 ¥	**SMAD4 probe** 11841-L29917		**Exon 9**		
252 *	Reference probe 20527-L28117	1q31			
258 ¥	**BMPR1A probe** 22025-L30948			**Exon 4**	
266	**SMAD4 probe** 05142-L07337		**Exon 3**		
275	**BMPR1A probe** 05131-L07338			**Exon 3**	
285	**BMPR1A probe** 07191-L07339			**Exon 3**	
292	Reference probe 03796-L03237	21q22			
301	**SMAD4 probe** 11842-L12639		**Exon 10**		
310 ±	**BMPR1A probe** 11843-L12640			**Exon 7**	
319	**PTEN probe** 03639-L02946				**Exon 6**
328	**BMPR1A probe** 07196-L06811			**Exon 9**	
337	**BMPR1A probe** 07199-L06814			**Exon 12**	
346	**SMAD4 probe** 05143-L04533		**Exon 4**		
355	**BMPR1A probe** 19659-L26347			**Exon 5**	
362	**SMAD4 probe** 07799-L26846		**Exon 11**		
370	Reference probe 08326-L22797	17q11			
376 ¥	**PTEN probe** 03638-L25975				**Exon 5**
382	**BMPR1A probe** 11844-L19281			**Exon 6**	
391	Reference probe 11958-L19280	20p12			
397 ¥	**PTEN probe** 21288-L02947				**Exon 8**
409 ¥	**SMAD4 probe** 16522-L31346		**Exon 5**		
418 «	**SMAD4 probe** 07797-L19282		**Exon 1**		
427	**SMAD4 probe** 07800-L07555		**Exon 12**		
436	**PTEN probe** 16651-L19278				**Exon 9**
445	**SMAD4 probe** 05145-L07344		**Exon 6**		
454	**BMPR1A probe** 05138-L07343			**Exon 13**	
463 «	**SMAD4 probe** 07798-L07553		**Exon 1**		
472 ¥	**PTEN probe** 17394-L29893				**Exon 1**
481	**PTEN probe** 16652-L19185				**Exon 3**
490	Reference probe 14909-L17529	18p11			
499 «	Reference probe 14882-L21050	14q11			

* New in version D1 (from lot D1-0918 onwards).
¥ Changed in version D1 (from lot D1-0918 onwards). Small change in length, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
± SNP rs7920259 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Note: The exon numberings used in this P158-D1 JPS product description are the exon numberings from the RefSeq transcripts NM_005359.5, NM_004329.2, and NM_000314.4, which are identical to the LRG_318, LRG_298, and LRG_311 sequences respectively. The exon numberings and NM sequences used are from 10/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. P158-D1 probes arranged according to chromosomal location

Table 2a. *SMAD4*

Length (nt)	SALSA MLPA probe	*SMAD4* exon	Ligation site NM_005359.5	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*539-541 (exon 2)*		
124 «	S0151-L14963	Exon 1	249 nt before exon 1	CCTTGGATACTT-TTTTGCAACGAG	0.3 kb
166 «	07796-L08332	Exon 1	32-33	AAGTTGGCAGCA-ACAACACGGCCC	0.4 kb
463 «	07798-L07553	Exon 1	3 nt after exon 1	GAGCCCAGGTAA-CCGCGCCATGTC	0.2 kb
418 «	07797-L19282	Exon 1	242 nt after exon 1	GCTCGTGGGAGA-ATCAAGTTAAAC	16.2 kb
142 *	02127-L01638	Exon 2	602-603	GCATTGTGCATA-GTTTGATGTGCC	1.6 kb
266 *	05142-L07337	Exon 3	814-815	GGATTTCCTCAT-GTGATCTATGCC	0.6 kb
346	05143-L04533	Exon 4	24 nt after exon 4	TTCTTACTACTT-TCTCTTTGTTTT	5.6 kb
409 *	16522-L31346	Exon 5	1115-1116	GTGCATCGACAG-AGACATACAGCA	3.3 kb
445 *	05145-L07344	Exon 6	1257-1258	ACTGTTGCAGAT-AGCATCAGGGCC	0.2 kb
238	21420-L30664	Exon 7	1336-1337	GACAGCACTACC-ACCTGGACTGGA	1.5 kb
196 *	05147-L07333	Exon 8	1466-1467	ATGAGCTTGCAT-TCCAGCCTCCCA	5.6 kb
244 *	11841-L29917	Exon 9	1536-1537	TGAAATGGATGT-TCAGGTAGGAGA	1.6 kb
301 *	11842-L12639	Exon 10	1707-1708	TGTGCAGTTGGA-ATGTAAAGGTGA	9.6 kb
362 *	07799-L26846	Exon 11	1878-1879	TCATCGACAGAT-GCAGCAGCAGGC	1.7 kb
427 *	07800-L07555	Exon 12	2098-2099	AGCATCAAAGAA-ACACCTTGCTGG	
		stop codon	*2195-2197 (exon 12)*		

Table 2b. *BMPR1A*

Length (nt)	SALSA MLPA probe	*BMPR1A* exon	Ligation site NM_004329.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*549-551 (exon 3)*		
222 «#	11840-L31344	Exon 1	30-31	GGAATCCGCCTG-CCGGGCTTGGCG	0.3 kb
137 «	07189-L15131	Exon 1	5 nt after exon 1	GTCCGGGTGAGT-TGGGAGTGCGCG	82.0 kb
218	21424-L29928	Exon 2	386-387	TTTATCTAGCCA-CATCTTGGAGGT	37.0 kb
275 #	05131-L07338	Exon 3	510-511	AAGACCAATTAT-TAAAGGTGACAG	0.1 kb
285	07191-L07339	Exon 3	601-602	TTTGTTCATCAT-TTCTCGTGTTCA	14.1 kb
258 #	22025-L30948	Exon 4	725-726	TTGCCTTTTTTA-AAGTGCTATTGC	2.1 kb
355	19659-L26347	Exon 5	3 nt after exon 5, reverse	CAAATTATATCT-TACTTTGCACTG	7.7 kb
382	11844-L19281	Exon 6	17 nt after exon 6	TAGCCGAGAAAA-GTCGGAGCATGC	0.1 kb
310 ±	11843-L12640	Exon 7	44 nt before exon 7	ACACGTCAGATT-ATTTTTTCATTT	12.4 kb
172	16647-L19180	Exon 8	52 nt after exon 8	TAGAATGTGTCC-TCATGATGGTGG	4.9 kb
328	07196-L06811	Exon 9	1412-1413	CATGAAAACATA-CTTGGTGGGTAC	1.8 kb
160	07197-L06812	Exon 10	1419-1418, reverse	TGCCGCTATGAA-ACCTGTCCAGTT	2.3 kb
211	16649-L19182	Exon 11	5 nt before exon 11, reverse	GTGTCACTGAAA-CAAAAGAAAGCC	2.0 kb
337	07199-L06814	Exon 12	8 nt after exon 12	GAAGTGAGTGGA-ACTCAGTCCCCT	0.6 kb
454 #	05138-L07343	Exon 13	2518-2519	AGATAAATGAGC-GCAGCAGAGATG	
		stop codon	*2145-2147 (exon 13)*		

Table 2c. *PTEN*

Length (nt)	SALSA MLPA probe	*PTEN* exon	Ligation site NM_000314.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*1032-1034 (exon 1)*		
184	11280-L15522	Promoter	1663 nt before exon 1	CAACATCGGAGA-ATGCACGCTCTG	2.8 kb
472	17394-L29893	Exon 1	5 nt after exon 1	TTGACCTGTATC-CATTTCTGCGGC	29.8 kb
190	06729-L06339	Exon 2	217 nt after exon 2, reverse	TATCACATAAGT-ACCTGATTATGT	31.3 kb
481	16652-L19185	Exon 3	29 nt after exon 3	TTTGTATGCTTG-CAAATATCTTCT	0.1 kb
178	16648-L19181	Exon 3	157 nt after exon 3	AGAGCATTGTTA-GATCATTTAGAA	5.4 kb
229	03718-L02944	Exon 4	14 nt after exon 4, reverse	ACATAGTACAGT-ACATTCATACCT	2.1 kb
376 #	03638-L25975	Exon 5	1436-1437	GGTGTAATGATA-TGTGCATATTTA	19.1 kb
319	03639-L02946	Exon 6	1659-1658, reverse	CTTACTGCAAGT-TCCGCCACTGAA	5.7 kb
148 #	19343-L25668	Exon 7	1703-1704	AAGGTGAAGATA-TATTCCTCCAAT	3.3 kb
397 #	21288-L02947	Exon 8	45 nt after exon 8	GACTTGTATGTA-TGTGATGTGTGT	6.9 kb
436	16651-L19278	Exon 9	4862-4863	ATGTCTGAAGTT-ACTTGAAGGCAT	
		stop codon	*2241-2243 (exon 9)*		

« 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.
± SNP rs7920259 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
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.
* A recurrent duplication of exons 2, 3 and 5-12 has been reported (ACMG posters Myriad & InVitae, 2015; Millson et al. (2015) *J Mol Diagnostics*). This is due to a processed SMAD4 pseudogene which is present in ~0.25% of the population. This pseudogene probably has no clinical significance. In such samples, the 346nt 05143-L04533 and the 238nt 21420-L30664 probes are not duplicated.

Note: The exon numberings used in this P158-D1 JPS product description are the exon numberings from the RefSeq transcripts NM_005359.5, NM_004329.2, and NM_000314.4, which are identical to the LRG_318, LRG_298, and LRG_311 sequences respectively. The exon numberings and NM sequences used are from 10/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

P003 MLH1/MSH2	Contains probes for the *MLH1* and *MSH2* genes
P067 PTCH1	Contains probes for the *PTCH1* gene
P072 MSH2-MUTYH	Contains probes for the *MSH2* and *MUTYH* genes
P093 HHT/PPH1	Contains probes for the *ENG* gene, which has been found to cause JPS in rare cases.
P101 STK11	Contains probes for the *STK11* gene
P225 PTEN	Contains more probes for the *PTEN* gene.
P378 MUTYH	Contains probes for the *MUTYH* gene
P472 SUFU	Contains probes for the *SUFU* gene

References

- Mancini et al. (2015). Dosage analysis by next generation sequencing and microarray CGH indicates putative processed pseudogenes in SMAD4 and NBN. *Presented at ACMG 2015* (also see https://www.myriadpro.com/for-your-practice/myriad-publications/).
- Millson A et al. (2015). Processed pseudogene confounding deletion/duplication analysis assays for SMAD4. *J Mol Diagnostics 17:1-7.*
- Patil et al. (2015). Discovery and clinical interpretation of a SMAD4 retrotransposon using next-generation sequencing. *Presented at ACMG 2015* (also see https://www.invitae.com/en/presentations/).
- 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.

Selected publications using SALSA MLPA Probemix P158 JPS

- Aretz S et al. (2007). High proportion of large genomic deletions and a genotype phenotype update in 80 unrelated families with juvenile polyposis syndrome. *J Med Genet.* 44:702-9.
- Calva-Cerqueira D et al. (2009). The rate of germline mutations and large deletions of SMAD4 and BMPR1A in juvenile polyposis. *Clin Genet.* 75:79-85.
- Cheah PY et al. (2009). Germline bone morphogenesis protein receptor 1A mutation causes colorectal tumorigenesis in hereditary mixed polyposis syndrome. *Am J Gastroenterol.* 104:3027-3.
- Menko FH et al. (2008). Variable phenotypes associated with 10q23 microdeletions involving the PTEN and BMPR1A genes. *Clin Genet.*, 74(2), 145-154.
- Millson A et al. (2015). Processed pseudogene confounding deletion/duplication analysis assays for SMAD4. J Mol Diagnostics 17:1-7.
- Ravegnini G et al. (2018). Gastrointestinal juvenile-like (inflammatory/hyperplastic) mucosal polyps in neurofibromatosis type 1 with no concurrent genetic or clinical evidence of other syndromes. *Virchows Archiv*, 1-6.
- Van Hattem WA et al. (2008). Large genomic deletions of SMAD4, BMPR1A and PTEN in juvenile polyposis. *Gut* 57:623-7.

P158 Product history	
Version	*Modification*
D1	One *BMPR1A* and *SMAD4* probe replaced, one *PTEN* probe added, one *SMAD4* flanking probe removed and two reference probes replaced and one added. Also, the length of several probes has been adjusted.
C2	Three reference probes have been replaced.
C1	Four *PTEN* probes, two *BMPR1A* probes and five reference probes have been replaced or included.
B1	Four *BMPR1A* probes, one *PTEN* probe, two *SMAD4* probes and four reference probes have been replaced or included. In addition, extra control fragments at 100 and 105 nt (X/Y specific) have been added. Probes for all *BMPR1A*, *SMAD4* and *PTEN* are now present.
A1	First release.

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

Implemented changes in the product description

Version D1-01 – 01 November 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).
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 20 – 11 August 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 2, new picture included).
- Various minor textual changes.

Version 19 – 29 February 2016 (55)
- Product description adapted to a new lot (lot number added, new picture included).
- Various minor layout changes.

Version 18 – 21 August 2015 (54)
- Extra information about a novel SMAD4 pseudogene and a SMAD4 retrotransposon added on page 1.

Version 17 – 18 May 2015 (54)
- New information about a novel SMAD4 pseudogene and a SMAD4 retrotransposon added on page 1.

Version 16 (53)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).

Version 15 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.