Product Description
SALSA® MLPA® Probemix P158-D1 JPS

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 10.

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 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 P158 JPS is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *BMPR1A* and *SMAD4* genes, as well as the 10q22-q23 microdeletion, which contains both *BMPR1A* and *PTEN*, in genomic DNA isolated from human peripheral whole blood specimens. P158 JPS is intended to confirm a potential cause for and clinical diagnosis of Juvenile Polyposis Syndrome (JPS) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P158 JPS should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *BMPR1A*, *SMAD4* and *PTEN* genes are point mutations, none of which will be detected by MLPA. It is therefore 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, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[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 and Coffalyser.Net analysis software.

Clinical background
Juvenile Polyposis Syndrome (JPS) is characterized by predisposition to hamartomatous polyps (non-malignant tumours, composed of tissue elements normally found at that site) in the gastrointestinal (GI) tract, specifically in the stomach, small intestine, colon, and rectum. The term "juvenile" refers to the type of polyp rather than to the age of onset of polyps. Most individuals with JPS have some polyps by the age of 20 years; some may have only four or five polyps over their lifetime, whereas others in the same family may have more than a hundred. If the polyps are left untreated, they may cause bleeding and anaemia. Most juvenile polyps are benign; however, malignant transformation can occur. Risk for GI cancers in families with JPS ranges from 9% to 50%. Most of this increased risk is attributed to colon cancer, but cancers of the stomach, upper GI tract, and pancreas have also been reported (NCBI GeneReviews: Juvenile Polyposis Syndrome).

Defects in the genes bone morphogenetic protein receptor type 1A (*BMPR1A*) and SMAD family member 4 (*SMAD4*) account for up to 60% of cases with JPS, with approximately 27% caused by defects in *SMAD4* and 24% in *BMPR1A* (Aretz et al. 2007, Calva-Cerqueira et al. 2009, van Hattem et al. 2008). There are reports of two other genes wherein defects could cause JPS, namely endoglin (*ENG)* and phosphatase and tensin homolog (*PTEN*). However, to date, defects in *ENG* have only been reported in two cases and defects in *PTEN* are thought to underlie Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome, also associated with juvenile polyps (collectively referred to as *PTEN* hamartoma tumor syndrome), instead of JPS. Lastly, 10q22-q23 microdeletion, involving complete deletions of *BMPR1A* and *PTEN* have been described to underlie JPS. However, the reports on the resulting phenotype are inconsistent. In some cases it leads to a severe, early onset form of juvenile polyposis. In other cases it leads to a syndrome suggestive of Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome.

A combined syndrome of JPS and hereditary haemorrhagic telangiectasia (HHT) (termed JPS/HHT) is thought to be present in most individuals with a *SMAD4* pathogenic variant. Studies have suggested that 15-22% of individuals with a pathogenic variant in *SMAD4* have this combined type, however this is thought to be an underestimation. While symptoms of JPS are limited to the GI tract, HHT characteristic arterial malformations can, beside the GI tract, be found in the lungs, liver, brain and mucocutaneous tissue. Upon detection of polyps in the GI tract, the arterial malformations are sometimes overlooked by physicians.

An investigation on *SMAD4* supports the presence of a pseudogene in a small percentage of individuals, 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).

Gene structure
The *BMPR1A* gene (13 exons) spans ~171 kb of genomic DNA and is located on chromosome 10q23.2, about 89 Mb from the p-telomere. The *BMPR1A* LRG_298 is identical to GenBank NG_009362 and is available at www.lrg-sequence.org.

The *SMAD4* gene (12 exons) spans ~55 kb of genomic DNA and is located on chromosome 18q21.2, about 47 Mb from the p-telomere. The *SMAD4* LRG_318 is identical to GenBank NG_013013 and is available at www.lrg-sequence.org.

The *PTEN* gene (9 exons) spans ~108 kb of genomic DNA and is located on chromosome 10q23.31, about 90 Mb from the p-telomere. The *PTEN* LRG_311 is identical to GenBank NG_007466 and is available at www.lrg-sequence.org.

Transcript variants
One *BMPR1A* transcript variant has been described encoding the full length protein (NM_004329.3; 6417 nt, coding sequence 569-2167; https://www.ncbi.nlm.nih.gov/gene/657). The ATG translation start site is in exon 3 and the stop codon in exon 13.

One *SMAD4* transcript variant has been described encoding the full length protein (NM_005359.6; 8772 nt, coding sequence 539-2197; https://www.ncbi.nlm.nih.gov/gene/4089). The ATG translation start site is in exon 2 and the stop codon in exon 12.

Three *PTEN* transcript variants have been described of which NM_000314.8; (8515 nt, coding sequence 846-2057; https://www.ncbi.nlm.nih.gov/gene/5728) is the most abundant. The ATG translation start site is in exon 1 and the stop codon in exon 9. Transcript variant *PTEN*-L (NM_001304717.5) is derived from the use of an upstream non-AUG (CUG) start codon and is the longest isoform. The third variant (NM_001304718.2) contains and lacks alternate exons in its 5' UTR compared to the other variants (variant 1).

Exon numbering
The exon numbering used in this P158-D1 JPS product description for gene *BMPR1A* is the exon numbering from the LRG_298 sequence; for gene *SMAD4* is the exon numbering from the LRG_318 sequence; for gene *PTEN* is the exon numbering from the LRG_311 sequence. 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 P158-D1 JPS contains 51 MLPA probes with amplification products between 118 and 499 nucleotides (nt). This includes 15 probes for the all 13 exons of the *BMPR1A* gene, with two additional probes each for exons 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 that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available 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).

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 human peripheral whole blood specimens, 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
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 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 (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 number(s) NA07891 (heterozygous deletion of the complete *SMAD4* gene), NA01359 (heterozygous duplication of the complete *SMAD4* gene), NA03623 (heterozygous duplication of the complete *SMAD4* gene) and NA20125 (heterozygous duplication of the complete *BMPR1A* and *PTEN* gene) from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control sample. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P158-D1	Expected copy number alteration
NA07891	Coriell Institute	18q21.2	*SMAD4*	heterozygous deletion
NA01359	Coriell Institute	18q21.2	*SMAD4*	heterozygous duplication
NA03623	Coriell Institute	18q21.2	*SMAD4*	heterozygous duplication
NA20125	Coriell Institute	10q23.2 & 10q23.31	*BMPR1A* and *PTEN*	heterozygous duplication

* 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 P158-D1 JPS probemix.

Performance characteristics

Many JPS patients have pathogenic variants that are easily detectable by sequence analysis. It is estimated that 4-15% of JPS patients have large rearrangements in the *BMPR1A* or *SMAD4* gene, either involving part of the gene, the whole gene or a larger microdeletion involving also the *PTEN* gene. When MLPA is used in addition to sequence analysis of the *BMPR1A* and *SMAD4* genes, the detection rate generally increases by ~25%.

The analytical sensitivity and specificity (based on a 2006-2021 literature review) for the detection of deletions in *BMPR1A*, *SMAD4* and *PTEN* is very high and can be considered >99%.

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 the *BMPR1A*, *SMAD4* and *PTEN* probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication).

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria 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	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.

- 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. 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 *BMPR1A* and *SMAD4* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *BMPR1A*, *SMAD4* and *PTEN* genes 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. 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.

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.

Mutation databases

BMPR1A mutation database https://databases.lovd.nl/shared/genes/*BMPR1A*;
SMAD4 mutation database https://databases.lovd.nl/shared/genes/*SMAD4*;
PTEN mutation database https://databases.lovd.nl/shared/genes/*PTEN*.
We strongly encourage users to deposit positive results in these respective databases. 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 SNVs and unusual results (e.g., a duplication of *BMPR1A* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P158-D1 JPS

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*SMAD4*	*BMPR1A*	*PTEN*
64-105	Control fragments – see table in probemix content section for more information				
118 *	Reference probe 19041-L24884	5q			
124 «	**SMAD4 probe** S0151-L14963		**Exon 1**		
130	Reference probe 00797-L00463	5q			
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	15q			
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	7q			
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	1q			
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	21q			
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	17q			
376 ¥	**PTEN probe** 03638-L25975				**Exon 5**
383	**BMPR1A probe** 11844-L19281			**Exon 6**	
391	Reference probe 11958-L19280	20p			
400 ¥	**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	18p			
499 ‡	Reference probe 14882-L21050	14q			

[a] See section Exon numbering on page 3 for more information.

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
‡ When present, SNP rs147586703 influences the probe signal (signal reduction).
‡‡ When present, SNP rs146326040 influences the probe signal (signal reduction).
« 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.

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. P158-D1 probes arranged according to chromosomal location

Table 2a. *BMPR1A*

Length (nt)	SALSA MLPA probe	*BMPR1A* exon[a]	Ligation site NM_004329.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*569-571 (exon 3)*		
222 #«	11840-L31344	Exon 1	50-51	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	406-407	TTTATCTAGCCA-CATCTTGGAGGT	37.0 kb
275 #	05131-L07338	Exon 3	530-531	AAGACCAATTAT-TAAAGGTGACAG	0.1 kb
285	07191-L07339	Exon 3	621-622	TTTGTTCATCAT-TTCTCGTGTTCA	14.1 kb
258 #	22025-L30948	Exon 4	745-746	TTGCCTTTTTTA-AAGTGCTATTGC	2.1 kb
355	19659-L26347	Exon 5	3 nt after exon 5, reverse	CAAATTATATCT-TACTTTGCACTG	7.7 kb
383	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	1432-1433	CATGAAAACATA-CTTGGTGGGTAC	1.8 kb
160	07197-L06812	Exon 10	1439-1438, 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	2538-2539	AGATAAATGAGC-GCAGCAGAGATG	
		stop codon	*2165-2167 (exon 13)*		

Table 2b. *PTEN*

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_000314.8	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*846-848 (exon 1)*		
184	11280-L15522	Promoter	1850 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	1250-1251	GGTGTAATGATA-TGTGCATATTTA	19.1 kb
319	03639-L02946	Exon 6	1473-1472, reverse	CTTACTGCAAGT-TCCGCCACTGAA	5.7 kb
148 #	19343-L25668	Exon 7	1517-1518	AAGGTGAAGATA-TATTCCTCCAAT	3.3 kb
400 #	21288-L02947	Exon 8	45 nt after exon 8	GACTTGTATGTA-TGTGATGTGTGT	6.9 kb
436	16651-L19278	Exon 9	4676-4677	ATGTCTGAAGTT-ACTTGAAGGCAT	
		stop codon	*2055-2057 (exon 9)*		

Table 2c. *SMAD4*

Length (nt)	SALSA MLPA probe	*SMAD4* exon[a]	Ligation site NM_005359.6	Partial sequence[b] (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)*		

[a] See section Exon numbering on page 3 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.

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 *SMAD4* exons has been reported (Millson et al. 2015; ACMG posters Myriad & InVitae, 2015). This is due to a processed *SMAD4* pseudogene which is present in ~0.25% of the population that affects exons 2, 3, 5, 6 and 8-12 probe ratios. This pseudogene probably has no clinical significance. In such samples, the 346 nt 05143-L04533 (exon 4) and the 238 nt 21420-L30664 (exon 7) probes are not duplicated.
« 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.

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.

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- Van Hattem WA et al. (2008). Large genomic deletions of SMAD4, BMPR1A and PTEN in juvenile polyposis. Gut 57:623-7.

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- 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), 145-54.
- 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.

P158 product history	
Version	*Modification*
D1	One *BMPR1A* and one *SMAD4* probe have been replaced, one *PTEN* probe has been added, one *SMAD4* flanking probe has been removed, two reference probes have been replaced and one has been 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.

Implemented changes in the product description
Version D1-04 – 22 November 2023 (04P) - Added warning for SNP rs146326040. - Length for BMPR1A probe 11844-L19281 and PTEN probe 21288-L02947 adjusted. Version D1-03 – 07 July 2021 (04P) - Ligation sites of the probes targeting the *BMPR1A*, *SMAD4* and *PTEN* genes updated according to new version of the NM_ reference sequence. - Removed warning concerning SNP rs7920259. - Added warning for salt sensitive probes. - Product description adapted to a new template. - UK added to the list of European countries that accept the CE-mark. - Intended purpose updated. *Version D1-02 – 11 December 2019 (02P)* - Product description restructured and adapted to a new template. - P158-D1 is now CE-marked.

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	EUROPE* CE
	ALL OTHER COUNTRIES

* comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.