Product Description
SALSA® MLPA® Probemix P148-C1 TGFBR1-TGFBR2-TGFB2-SMAD3

To be used with the MLPA General Protocol.

Version C1
As compared to version B4, 15 target probes have been added (targeting *TGFB2* and *SMAD3*), one TGFBR1 probe has been removed, five reference probes have been replaced, and nine target probes and one reference probe have been changed in length but no changes in sequence detected. For complete product history see page 9.

Catalogue numbers
- **P148-025R:** SALSA® MLPA® Probemix P148 TGFBR1-TGFBR2-TGFB2-SMAD3, 25 reactions
- **P148-050R:** SALSA® MLPA® Probemix P148 TGFBR1-TGFBR2-TGFB2-SMAD3, 50 reactions
- **P148-100R:** SALSA® MLPA® Probemix P148 TGFBR1-TGFBR2-TGFB2-SMAD3, 100 reactions

SALSA® MLPA® Probemix P148 TGFBR1-TGFBR2-TGFB2-SMAD3 (hereafter: P148 TGFBR1-TGFBR2-TGFB2-SMAD3) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P148-025R	**P148-050R**	**P148-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding 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

SALSA® MLPA® Probemix P148 TGFBR1-TGFBR2-TGFB2-SMAD3 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *TGFBR1*, *TGFBR2*, *TGFB2* and *SMAD3* genes, which are associated with Loeys-Dietz syndrome (LDS). LDS is a connective tissue disorder that can manifest in a variety of ways, amongst others with vascular findings, skeletal manifestations, craniofacial features and cutaneous findings. The severity and nature of symptoms can vary greatly between affected individuals, with significant extravascular systemic findings in young children to predominantly thoracic aortic aneurysm/dissection occurring in adults. LDS individuals were first described with a Marfan syndrome-like phenotype and LDS has some features in common with Marfan syndrome, however LDS and Marfan syndrome are distinct syndromes and have different genetic causes. Pathogenic variants in multiple genes are associated with LDS. The highest proportion of pathogenic variants is found in *TGFBR2*, followed by *TGFBR1*, *TGFB2* and *SMAD3*.

The *TGFBR1* gene (9 exons) spans ~49 kb of genomic DNA and is located on chromosome 9q22.33, ~101 Mb from the p-telomere. The *TGFBR2* gene (7 exons) spans ~88 kb of genomic DNA and is located on chromosome 3p24.1, ~31 Mb from the p-telomere. The *TGFB2* gene (7 exons) spans ~99 kb and is located on chromosome 1q41, ~217 Mb from the p-telomere. The *SMAD3* gene (9 exons) spans ~130 kb and is located on chromosome 15q22.33, ~65 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1133/.

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:

Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE
Tark – Transcript Archive: https://tark.ensembl.org

Exon numbering

The *TGFBR1*, *TGFBR2*, *TGFB2* and *SMAD3* exon numbering used in this P148-C1 TGFBR1-TGFBR2-TGFB2-SMAD3 product description is the exon numbering derived from MANE project (release version 1.0) based on MANE Select transcript NM_004612.4, NM_003242.6, NM_003238.6 and NM_005902.4. The *TGFBR2* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version C1-01 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select NM_ sequence for this gene. 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

P148-C1 TGFBR1-TGFBR2-TGFB2-SMAD3 contains 44 MLPA probes with amplification products between 129 and 490 nucleotides (nt). This includes ten probes for *TGFBR1*, nine probes for *TGFBR2*, six probes for *TGFB2* and nine probes for *SMAD3*. 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 MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of Loeys-Dietz syndrome (LDS) and Marfan syndrome. It is required to use samples of the same sex to facilitate interpretation. 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. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 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/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 Coffalyser.Net (calculations, cut-offs and interpretation remain unchanged). Please note that Coffalyser.Net 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. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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 *TGFBR1* 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 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 *TGFBR1*, *TGFBR2*, *TGFB2* and *SMAD3* genes are small (point) mutations, none of which will be detected by using P148 TGFBR1-TGFBR2-TGFB2-SMAD3.
- 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 in sequence data 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.

***TGFBR1*, *TGFBR2*, *TGFB2* and *SMAD3* mutation database**

https://databases.lovd.nl/shared/genes/TGFBR1
https://databases.lovd.nl/shared/genes/TGFBR2
https://databases.lovd.nl/shared/genes/TGFB2
https://databases.lovd.nl/shared/genes/SMAD3

We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://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 *TGFBR1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. P148-C1 TGFBR1-TGFBR2-TGFB2-SMAD3

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*TGFBR1*	*TGFBR2*	*TGFB2*	*SMAD3*
64-105	Control fragments – see table in probemix content section for more information					
129 *	Reference probe 19616-L26684	4p				
136 *	**SMAD3 probe** 23376-L33035					**Exon 1**
142 *	**TGFB2 probe** 23385-L33044				**Exon 5**	
150 *	**SMAD3 probe** 23377-L33036					**Exon 4**
154	Reference probe 12416-L13417	22q				
160 «	**TGFBR1 probe** 04644-L04028		upstream			
166 *	**TGFB2 probe** 23386-L33137				**Exon 1**	
172	**TGFBR2 probe** 02797-L06029			**Exon 7**		
177 *	**SMAD3 probe** 23378-L33037					**Exon 7**
184 «	**TGFBR1 probe** 04643-L04027		upstream			
196 ¥	**TGFBR2 probe** 03861-L03610			**Exon 2**		
202	Reference probe 18560-L24870	8q				
206 *	**SMAD3 probe** 23379-L33038					**Exon 8**
214	**TGFBR1 probe** 19509-L25943		**Exon 2**			
220 *	**TGFB2 probe** 23387-L33046				**Exon 2**	
226	**TGFBR2 probe** 22015-L03895			**Exon 3**		
232 *	**SMAD3 probe** 23380-L33039					**Exon 5**
241 ¥	**TGFBR1 probe** 04648-L33066		**Exon 3**			
247	Reference probe 21928-L30731	15q				
254 ¥	**TGFBR2 probe** 23394-L03246			**Exon 4**		
264 ¥	**TGFBR1 probe** 23395-L04034		**Exon 5**			
274 *	Reference probe 18667-L24021	11p				
281 *	**SMAD3 probe** 23381-L33040					**Exon 3**
289 ¥	**TGFBR1 probe** 04649-L33068		**Exon 4**			
298 ¥	**TGFBR2 probe** 03864-L33069			**Exon 5**		
304 ¥	Reference probe 22146-L33070	16p				
310 ¥	**TGFBR1 probe** 04651-L33071		**Exon 6**			
319	**TGFBR1 probe** 04652-L04036		**Exon 7**			
328	**TGFBR2 probe** 03865-L03248			**Exon 6**		
337 *	Reference probe 17370-L21036	20q				
346 *	**TGFB2 probe** 23388-L33047				**Exon 7**	
358 ¥	**TGFBR1 probe** 05078-L33072		**Exon 9**			
364	**TGFBR2 probe** 02796-L02181			**Exon 3**		
383 ±	**TGFBR2 probe** 02795-L28027			**Exon 1**		
393	Reference probe 19791-L26597	1q				
400	**TGFBR2 probe** 04665-L14635			**Exon 1**		
409 *	**SMAD3 probe** 23382-L33041					**Exon 9**
418 ¥	**TGFBR1 probe** 04653-L33073		**Exon 8**			
436 *	Reference probe 04279-L23590	12q				
454 *	**TGFB2 probe** 23390-L33049				**Exon 6**	
463 *	**SMAD3 probe** 23383-L33042					**Exon 2**
474 *	**TGFB2 probe** 23391-L33050				**Exon 4**	
483 *	**SMAD3 probe** 23384-L33043					**Exon 6**
490 *	Reference probe 22744-L32038	14q				

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

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in sequence detected.
± SNV rs138010137 could influence the probe signal. 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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation 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. Target and flanking probes arranged according to chromosomal location

Table 2a. *TGFBR1*

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b] NM_004612.4	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
184 «	04643-L04027	upstream	509 nt before exon 1	GGGGAAGTTGAA-GTGGATGCAGGG	0.1 kb
160 «	04644-L04028	upstream	387 nt before exon 1	GCGCCTAGAGGA-GGTTAGAAGAAA	24.2 kb
		start codon	*94-96 (Exon 1)*		
214	19509-L25943	Exon 2	214-215	TCTGCCACCTCT-GTACAAAAGACA	3.8 kb
241	04648-L33066	Exon 3	621-622	GAGGGTACTACG-TTGAAAGACTTA	5.2 kb
289	04649-L33068	Exon 4	737-738	TGGCAAAGGTCG-ATTTGGAGAAGT	4.6 kb
264	23395-L04034	Exon 5	936-937	TTGGTGTCAGAT-TATCATGAGCAT	2.3 kb
310	04651-L33071	Exon 6	1179-1180	GATTCAGCCACA-GATACCATTGAT	1.7 kb
319	04652-L04036	Exon 7	1251-1252	GTTCTCGATGAT-TCCATAAATATG	1.2 kb
418	04653-L33073	Exon 8	1392-1391 *reverse*	GATGGGTCAGAA-GGTACAAGATCA	1.6 kb
358	05078-L33072	Exon 9	1533-1534	GCCAATGGAGCA-GCTAGGCTTACA	
		stop codon	*1603-1605 (Exon 9)*		

Table 2b. *TGFBR2*

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b] NM_003242.6	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*284-286 (Exon 1)*		
383 ±	02795-L28027	Exon 1	9 nt before exon 1	TGAGTTGAAGTT-GAGTGAGTCACT	0.2 kb
400	04665-L14635	Exon 1	230-231	GGGGTCCGGGAA-GGCGCCGTCCGC	38.0 kb
196	03861-L03610	Exon 2 (3)	474-475	CTGTGACAACCA-GAAATCCTGCAT	5.5 kb
364	02796-L02181	Exon 3 (4)	581-582	CACTAGAGACAG-TTTGCCATGACC	0.1 kb
226 ‡	22015-L03895	Exon 3 (4)	663-662 *reverse*	CACCAGGCTTTT-TTTTTTCCTTCA	21.4 kb
254	23394-L03246	Exon 4 (5)	822-823	GGGAGTTGCCAT-ATCTGTCATCAT	2.5 kb
298	03864-L33069	Exon 5 (6)	1632-1633	TGTCTACTCCAT-GGCTCTGGTGCT	14.3 kb
328	03865-L03248	Exon 6 (7)	1755-1756	GAAGGACAACGT-GTTGAGAGATCG	3.0 kb
172	02797-L06029	Exon 7 (8)	1833-1834	GTGTGAGACGTT-GACTGAGTGCTG	
		stop codon	*1985-1987 (Exon 7)*		

Table 2c. *TGFB2*

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b] NM_003238.6	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*1367-1369 (Exon 1)*		
166	23386-L33137	Exon 1	1407-1408	GATCCTGCATCT-GGTCACGGTCGC	58.6 kb
220	23387-L33046	Exon 2	1843-1844	AACCCAAAAGCC-AGAGTGCCTGAA	29.1 kb
474	23391-L33050	Exon 4	2079-2078 *reverse*	TATTTGGGATGA-TGTAATTATTAG	1.7 kb
142	23385-L33044	Exon 5	2214-2215	ACATCTCCTGCT-AATGTTATTGCC	1.4 kb
454	23390-L33049	Exon 6	1 nt after exon 6 reverse	GCTGAACACTCA-CCCTGCTGTGCT	3.8 kb
346	23388-L33047	Exon 7	2557-2558	ATTGGCAAAACA-CCCAAGATTGAA	
		stop codon	*2609-2611 (Exon 7)*		

Table 2d. *SMAD3*

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b] NM_005902.4	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*554-556 (Exon 1)*		
136	23376-L33035	Exon 1	724-725	GCCATCACCACG-CAGAACGTCAAC	98.8 kb
463	23383-L33042	Exon 2	3 nt after exon 2	AGACACCAGGTA-TGCTGCCTGGCC	0.2 kb
281	23381-L33040	Exon 3	1023-1022 *reverse*	GGATGGAATGGC-TGTAGTCGTCCA	1.5 kb
150	23377-L33036	Exon 4	1135-1134 *reverse*	TGGTTCATCTGG-TGGTCACTGGTT	3.8 kb
232	23380-L33039	Exon 5	1199-1200	TGTCCCCAGCAC-ATAATAACTTGG	10.6 kb
483	23384-L33043	Exon 6	1221-1220 *reverse*	AGTAGGTAACTG-GCTGCAGGTCTG	3.6 kb
177	23378-L33037	Exon 7	1502-1501 *reverse*	ACAGTTGGGAGA-CTGGACAAAAAT	2.7 kb
206	23379-L33038	Exon 8	5 nt after exon 8	AGTACAGGTCAG-TTATGGGTGCTG	3.0 kb
409	23382-L33041	Exon 9	1801-1802	CAGATGGGCTCC-CCAAGCATCCGC	
		stop codon	*1829-1831 (Exon 9)*		

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNV rs138010137 could influence the probe signal. 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.
‡ Please note that this probe contains a stretch of ten Thymidine (T) residues. Polymerases have problems in copying such regions. As a result, the amplification product of this probe has a shoulder with a length one nt shorter. This ten-T stretch might be hypermutated in cancers, especially in colorectal tumours manifesting high-frequency microsatellite instability (MSI-H) (Jung et al. 2004). We have no information on the % signal reduction of this probe on mutated sequences having a longer or shorter T stretch.

SNVs located in the target sequence of a probe can influence probe hybridisation 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.

Related products
For related products, see the product page on our website.

References

- Jung B et al. (2004). Loss of activin receptor type 2 protein expression in microsatellite unstable colon cancers. *Gastroenterology*. 126:654-659.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
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- 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 P148 TGFBR1-TGFBR2-TGFB2-SMAD3

- Baetens M et al. (2011). Applying massive parallel sequencing to molecular diagnosis of Marfan and Loeys-Dietz syndromes. *Hum Mutat*. 32:1053-1062.
- Furtado LV et al. (2011). Characterization of large genomic deletions in the FBN1 gene using multiplex ligation-dependent probe amplification. *BMC Med Genet*. 12:119.
- Goudie DR et al. (2011). Multiple self-healing squamous epithelioma is caused by a disease-specific spectrum of mutations in TGFBR1. *Nat Genet*. 43:365-369.
- Li J et al. (2017). Gross deletions in FBN1 results in variable phenotypes of Marfan syndrome. *Clin Chim Acta*. 474:54-59.

- Nayak SS et al. (2021). Clinically relevant variants in a large cohort of Indian patients with Marfan syndrome and related disorders identified by next-generation sequencing. *Sci Rep*. 11:764.

P148 product history	
Version	*Modification*
C1	15 target probes have been added (targeting *TGFB2* and *SMAD3*), one target probe has been removed, five reference probes have been replaced, and nine target probes and one reference probe have been changed in length but no changes in sequence detected.
B4	Eight reference probes have been replaced and one probe length has been adjusted.
B3	One flanking probe has been removed.
B2	Four reference probes have been replaced and QDX2 fragments have been added.
B1	One less reliable *TGFBR1* probe has been removed, one reference probe has been replaced and extra control fragments at 88-96-100-105 nt have been included.
A1	First release.

Implemented changes in the product description

Version C1-01 – 22 April 2025 (05P)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Product name updated from "SALSA MLPA Probemix P148 TGFBR1-TGFBR2" to "SALSA MLPA Probemix P148 TGFBR1-TGFBR2-TGFB2-SMAD3".
- General information rewritten.
- Exon numbering of the *TGFBR2* gene has been changed.
- Ligation sites of the probes targeting the *TGFBR2* gene updated according to new version of the NM_ reference sequence (changed from NM_001024847.2 to NM_003242.6).
- Various minor textual or layout changes.

Version B4-01 – 01 July 2021 (04P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *TGFBR1* gene updated according to new version of the NM_ reference sequence.
- Warning added in Table 1 and 2 for possible influence of SNP rs138010137 on the 382 nt TGFBR2 exon 1 probe.

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