Product Description
SALSA® MLPA® Probemix P148-B4 TGFBR1-TGFBR2

To be used with the MLPA General Protocol.

Version B4
As compared to version B3, eight reference probes have been replaced and one probe length has been adjusted. For complete product history see page 7.

Catalogue numbers:
- **P148-025R:** SALSA MLPA Probemix P148 TGFBR1-TGFBR2, 25 reactions.
- **P148-050R:** SALSA MLPA Probemix P148 TGFBR1-TGFBR2, 50 reactions.
- **P148-100R:** SALSA MLPA Probemix P148 TGFBR1-TGFBR2, 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 P148 TGFBR1-TGFBR2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *TGFBR1* and *TGFBR2* genes, which are associated with Loeys-Dietz aortic aneurysm syndrome (LDS) type Ia and IIa. In addition to LDS, mutations in *TGFBR2* have also been associated with Marfan syndrome and various types of tumours.

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 (8 exons) spans ~88 kb of genomic DNA and is located on chromosome 3p24.1, ~31 Mb from the p-telomere.

TGFBR1 and *TGFBR2* encode a transmembrane serine/threonine kinase receptor (type 1 and 2) for transforming growth factor-beta (TGF-ß). TGF-ß receptor type 1 regulates cell cycle progression by a signalling mechanism that involves ligand binding (TGF-ß) and heterodimerization with TGF-ß receptor type 2, resulting in a complex that triggers signal transduction by activation of various proteins in the TGF-ß pathway.

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

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

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

Exon numbering
The *TGFBR1* and *TGFBR2* exon numbering used in this P148 TGFBR1-TGFBR2 product description is the exon numbering from the NG_007461.1 and LRG_779 sequences, respectively. 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 P148-B4 TGFBR1-TGFBR2 contains 30 MLPA probes with amplification products between 154 and 423 nucleotides (nt). This includes eleven probes for the *TGFBR1* gene, one probe for each exon and two probes upstream of the gene, and nine probes for the *TGFBR2* gene, one probe for each exon with the exception of exon 2 since it is only present in transcript variant 1 and two probes for exon 1 and 4. 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 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 Loeys-Dietz aortic aneurysm syndrome (LDS) type Ia and IIa and Marfan syndrome. 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 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.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	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. 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 *TGFBR1* and *TGFBR2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P148 TGFBR1-TGFBR2.
- 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.

***TGFBR1* and *TGFBR2* mutation database**

https://databases.lovd.nl/shared/genes/TGFBR1 and https://databases.lovd.nl/shared/genes/TGFBR2. 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 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 *TGFBR1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P148-B4 TGFBR1-TGFBR2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*TGFBR1*	*TGFBR2*
64-105	Control fragments – see table in probemix content section for more information			
154 *	Reference probe 12416-L13417	22q		
160 «	**TGFBR1 probe** 04644-L04028		**Upstream**	
166 *	Reference probe 12741-L21552	21q		
172	**TGFBR2 probe** 02797-L06029			**Exon 8**
178	Reference probe 03179-L02518	9p		
184 «	**TGFBR1 probe** 04643-L04027		**Upstream**	
193	**TGFBR2 probe** 03861-L03244			**Exon 3**
202 *	Reference probe 18560-L24870	8q		
214	**TGFBR1 probe** 19509-L25943		**Exon 2**	
226 ¥	**TGFBR2 probe** 22015-L03895			**Exon 4**
238	**TGFBR1 probe** 04648-L04032		**Exon 3**	
247 *	Reference probe 21928-L30731	15q		
256	**TGFBR2 probe** 03863-L03246			**Exon 5**
265	**TGFBR1 probe** 04650-L04034		**Exon 5**	
274 *	Reference probe 18336-L23249	14q		
283	**TGFBR1 probe** 04649-L04033		**Exon 4**	
292	**TGFBR2 probe** 03864-L03247			**Exon 6**
301 *	Reference probe 22146-L31174	16p		
308	**TGFBR1 probe** 04651-L04035		**Exon 6**	
319	**TGFBR1 probe** 04652-L04036		**Exon 7**	
328	**TGFBR2 probe** 03865-L03248			**Exon 7**
337 *	Reference probe 03264-L02701	3q		
355	**TGFBR1 probe** 05078-L04038		**Exon 9**	
364	**TGFBR2 probe** 02796-L02181			**Exon 4**
373 «	**TGFBR1 probe** 04645-L15171		**Exon 1**	
382 ±	**TGFBR2 probe** 02795-L28027			**Exon 1**
393 *	Reference probe 19791-L26597	1q		
400	**TGFBR2 probe** 04665-L14635			**Exon 1**
416	**TGFBR1 probe** 04653-L27799		**Exon 8**	
423	Reference probe 01058-L27800	17q		

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

* New in version B4.
¥ Changed in version B4. Minor alteration, no change in sequence detected.
± SNP 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P148-B4 probes arranged according to chromosomal location

Table 2a. *TGFBR1*

Length (nt)	SALSA MLPA probe	*TGFBR1* exon[a]	Ligation site NM_004612.4	Partial sequence[b] (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	0.4 kb
		start codon	*94-96 (Exon 1)*		
373 «	04645-L15171	Exon 1	27 nt before exon 1	CCTCCGAGCAGT-TACAAAGGGCCG	23.8 kb
214	19509-L25943	Exon 2	214-215	TCTGCCACCTCT-GTACAAAAGACA	3.8 kb
238	04648-L04032	Exon 3	621-622	GAGGGTACTACG-TTGAAAGACTTA	5.2 kb
283	04649-L04033	Exon 4	737-738	TGGCAAAGGTCG-ATTTGGAGAAGT	4.6 kb
265	04650-L04034	Exon 5	936-937	TTGGTGTCAGAT-TATCATGAGCAT	2.3 kb
308	04651-L04035	Exon 6	1179-1180	GATTCAGCCACA-GATACCATTGAT	1.7 kb
319	04652-L04036	Exon 7	1251-1252	GTTCTCGATGAT-TCCATAAATATG	1.2 kb
416	04653-L27799	Exon 8	1392-1391, reverse	GATGGGTCAGAA-GGTACAAGATCA	1.6 kb
355	05078-L04038	Exon 9	1533-1534	GCCAATGGAGCA-GCTAGGCTTACA	
		stop codon	*1603-1605 (Exon 9)*		

Table 2b. *TGFBR2*

Length (nt)	SALSA MLPA probe	*TGFBR2* exon[a]	Ligation site NM_001024847.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*383-385 (Exon 1)*		
382 ±	02795-L28027	Exon 1	90-91	TGAGTTGAAGTT-GAGTGAGTCACT	0.2 kb
400	04665-L14635	Exon 1	329-330	GGGGTCCGGGAA-GGCGCCGTCCGC	38.0 kb
	No probe	Exon 2			
193	03861-L03244	Exon 3	648-649	CTGTGACAACCA-GAAATCCTGCAT	5.5 kb
364	02796-L02181	Exon 4	755-756	CACTAGAGACAG-TTTGCCATGACC	0.1 kb
226 ‡	22015-L03895	Exon 4	837-836 reverse	CACCAGGCTTTT-TTTTTTCCTTCA	21.4 kb
256	03863-L03246	Exon 5	996-997	GGGAGTTGCCAT-ATCTGTCATCAT	2.5 kb
292	03864-L03247	Exon 6	1806-1807	TGTCTACTCCAT-GGCTCTGGTGCT	14.3 kb
328	03865-L03248	Exon 7	1929-1930	GAAGGACAACGT-GTTGAGAGATCG	3.0 kb
172	02797-L06029	Exon 8	2007-2008	GTGTGAGACGTT-GACTGAGTGCTG	
		stop codon	*2159-2161 (Exon 8)*		

[a] See section Exon numbering on page 1 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« 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 rs138010137 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
‡ 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 hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P065/P066 Marfan Syndrome	Contain probes for *FBN1* and *TGFBR2*. The probes for *TGFBR2* in this P148 probemix are identical to the *TGFBR2* probes in the SALSA MLPA probemixes P065/P066.

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.
- 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 P148 TGFBR1-TGFBR2

- 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.

P148 product history	
Version	*Modification*
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 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.

Version B3-01 – 05 July 2018 (01P)
- Product description restructured and adapted to a new template.
- Two references were removed, one new reference was added.

Version 11 – 01 February 2018 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes on page 1.
- New references added on page 2.
- Warning removed in table 1, for the probes 04665-L14635 and 02795-L28027.
- Ligation sites of the probes targeting the *TGFBR1* gene updated according to new version of the NM_reference sequence.

Version 10 - 22 April 2015 (54)
- Product description adapted to a new product version (version number changed, lot number changed, changes in Table 1 and Table 2, new picture included).
- Minor textual changes on page 1.

- Ligation sites of the probes targeting the *TGFBR2* gene updated according to the NM_reference sequence NM_001024847.2.

Version 09 (48)

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

More information: www.mrcholland.com; www.mrcholland.eu	
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