Product Description

SALSA® MLPA® Probemixes P065-C1 Marfan Syndrome-1 & P066-C1 Marfan Syndrome-2

To be used with the MLPA General Protocol.

P065 version C1
For complete product history see page 12.

P066 version C1
For complete product history see page 12.

Catalogue numbers:

- **P065-025R:** SALSA MLPA Probemix P065 Marfan Syndrome-1, 25 reactions.
- **P065-050R:** SALSA MLPA Probemix P065 Marfan Syndrome-1, 50 reactions.
- **P065-100R:** SALSA MLPA Probemix P065 Marfan Syndrome-1, 100 reactions.

- **P066-025R:** SALSA MLPA Probemix P066 Marfan Syndrome-2, 25 reactions.
- **P066-050R:** SALSA MLPA Probemix P066 Marfan Syndrome-2, 50 reactions.
- **P066-100R:** SALSA MLPA Probemix P066 Marfan Syndrome-2, 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 Probemixes P065 Marfan Syndrome-1 and P066 Marfan Syndrome-2 are in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assays[2] for the detection of deletions or duplications in the *FBN1* gene, in order to confirm a potential cause for and clinical diagnosis of Marfan syndrome and other *FBN1*-related disorders. P065 Marfan Syndrome-1 can also be used for the detection of deletions or duplications in the *TGFBR2* gene, in order to confirm a potential cause for and clinical diagnosis of *TGFBR2*-related disorders. Both assays are for use with genomic DNA isolated from human peripheral whole blood specimens and are also intended for molecular genetic testing of at-risk family members.

The detection of copy number variations (CNVs) in *FBN1* requires the use of both P065 Marfan Syndrome-1 and P066 Marfan Syndrome-2. CNVs detected with P065 Marfan Syndrome-1 and P066 Marfan Syndrome-2 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 *FBN1* and *TGFBR2* 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.

These devices are 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.

[1] Please note that these probemixes are for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the products are for research use only (RUO).
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background

Marfan syndrome is a systemic disorder of connective tissue that mainly affects the ocular, skeletal and cardiovascular systems. It has a penetrance of 100%, but a high degree of clinical variability, with phenotypes ranging from isolated features of Marfan syndrome to neonatal presentation of severe and progressive disease in multiple organ systems. The major causes of morbidity and mortality relate to the cardiovascular system. However, if proper management is executed, the life expectancy of a patient with Marfan syndrome approximates that of the general population. Marfan syndrome is an autosomal dominant disease with a prevalence of 1:5,000 – 1:10,000 that is caused by mutations in the *FBN1* gene (Dietz et al. 1991; Lee et al. 1991). In ~90-93% of patients diagnosed with Marfan syndrome a mutation is detected by sequencing of the *FBN1* coding region and flanking intronic regions. In ~5% of the patients, the pathogenic variant identified is a large deletion or duplication (Baetens et al. 2011; Hilhorst-Hofstee et al. 2011; Mannucci et al. 2020; Rand-Hendriksen et al. 2007; Stengl et al. 2020). In a small percentage of patients (~2-5%), no mutation in *FBN1* is identified. More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1335/.

Several Marfan-related disorders have been recognized that display a specific subset of the phenotypic features found in Marfan syndrome and that can also be caused by mutations in the *FBN1* gene. These *FBN1*-related disorders include MASS phenotype (mitral valve, aortic, skin and skeletal features), ectopia lentis syndrome and familial thoracic aortic aneurysm and dissection (familial TAAD). In addition, there are also Marfan-related disorders that are caused by mutations in *TGFBR2*. These *TGFBR2*-related disorders include Loeys-Dietz syndrome and familial TAAD. Loeys-Dietz syndrome is a systemic disorder of connective tissue that has a large overlap in clinical features with Marfan syndrome. The most common clinical features involve the vascular, skeletal, craniofacial, cutaneous, allergic/inflammatory and ocular systems. In ~55-60% of patients diagnosed with Loeys-Dietz syndrome a pathogenic variant in *TGFBR2* is identified. Sequence analysis detects ~100% of these pathogenic variants. Large rearrangements of *TGFBR2* have thus far not been reported in patients with Loeys-Dietz syndrome features. There is one report of a deletion encompassing the entire *TGFBR2* gene, but this individual thus far lacked aortic involvement and did not show clear features of Loeys-Dietz syndrome (Campbell et al. 2011). More information about Loeys-Dietz syndrome is available at https://www.ncbi.nlm.nih.gov/books/NBK1133/.

Gene structure

The *FBN1* gene spans ~238 kilobases (kb) on chromosome 15q21.1 and contains 66 exons. The *FBN1* LRG_778 is available at www.lrg-sequence.org and is identical to GenBank NG_008805.2.

The *TGFBR2* gene spans ~88 kb on chromosome 3p24.1 and contains 8 exons. The *TGFBR2* LRG_779 is available at www.lrg-sequence.org and is identical to GenBank NG_007490.1.

Transcript variants

For *FBN1,* one transcript variant has been described encoding the full length protein (NM_000138.5; 11609 nt; coding sequence 317-8932; https://www.ncbi.nlm.nih.gov/gene/2200). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2 and the stop codon is located in exon 66.

For *TGFBR2,* two transcript variants have been described (https://www.ncbi.nlm.nih.gov/gene/7048). Transcript variant 1 represents the longer transcript and encodes the longer isoform (NM_001024847.3; 4605 nt; coding sequence 284-2062). The ATG translation start site is located in exon 1 and the stop codon is located in exon 8. Transcript variant 2 (NM_003242.6; 4530 nt; coding sequence 284-1987) lacks an alternative in-frame exon in the coding region compared to variant 1, which results in a shorter isoform.

Exon numbering
The *FBN1* exon numbering used in this P065-C1/P066-C1 Marfan Syndrome product description is the exon numbering from the LRG_778 sequence. The *TGFBR2* exon numbering used in this product description is the exon numbering from the LRG_779 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 P065-C1 Marfan Syndrome-1 contains 52 MLPA probes with amplification products between 130 and 504 nucleotides (nt). The SALSA MLPA Probemix P066-C1 Marfan Syndrome-2 contains 49 MLPA probes with amplification products between 130 and 490 nt.

The P065-C1 and P066-C1 probemixes contain 34 probes and 36 probes for the *FBN1* gene, respectively. Together, these probemixes cover each exon of *FBN1* by at least one probe. Exon 2, 65 and 66 are covered by two probes and one probe upstream of *FBN1* is present. In addition, the P065-C1 probemix contains nine probes for the *TGFBR2* gene. Each exon of *TGFBR2* is covered by one probe, with the exception of exon 1, which is covered by two probes. The P065-C1 and P066-C1 probemixes contain nine and thirteen reference probes, respectively. These reference probes detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

These probemixes 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 blood, 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 Marfan syndrome and *FBN1*- and *TGFBR2*-related disorders. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers from the Coriell Institute described in the table below have been tested with the P065-C1 and P066-C1 probemixes at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID number	Source	Probemix by which CNV is detected	Expected CNV
NA03184	Coriell Institute	P065/P066	Heterozygous whole gene duplication of *FBN* (partial trisomy of chromosome 15)*
NA04127	Coriell Institute	P065	Heterozygous whole gene duplication of *TGFBR2* (partial trisomy of chromosome 3p)*
NA21939	Coriell Institute	P065/P066	Heterozygous deletion of *FBN1* exon 43-44
NA21940	Coriell Institute	P065/P066	Heterozygous deletion of *FBN1* exon 45-47

* The whole extent of the CNV present in this cell line cannot be determined by the P065-C1 Marfan Syndrome-1 and P066-C1 Marfan Syndrome-2 probemixes.

Performance characteristics
Deletions or duplications in *FBN1* are found in ~5% of Marfan syndrome patients (Baetens et al. 2011; Hilhorst-Hofstee et al. 2011; Mannucci et al. 2020; Rand-Hendriksen et al. 2007; Stengl et al. 2020; https://www.ncbi.nlm.nih.gov/books/NBK1335/). This percentage is, however, dependent on how strict the clinical criteria for Marfan syndrome are followed (Hung et al. 2009; Lerner-Ellis et al. 2014). The extent to which deletions or duplications in *FBN1* are involved in other *FBN1*-related disorders remains to be determined. Deletions and duplications in *TGFBR2* have thus far not been reported in Loeys-Dietz syndrome and other *TGFBR2*-related disorders (https://www.ncbi.nlm.nih.gov/books/NBK1133/). The analytical sensitivity and specificity for the detection of deletions or duplications in the *FBN1* and *TGFBR2* gene is very high and can be considered >99% (based on a 2006-2020 literature review).

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 *FBN1* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication) may be obtained. Although one whole gene deletion has been described for *TGFBR2* (Campbell et al. 2011), no other deletions or duplications have been found. However, theoretically allele copy numbers of 2, 1, 3 or 4 can be expected. A copy number of 0 cannot be expected for *TGFBR2* since a homozygous deletion is associated with embryonic lethality.

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 (Blyth et al. 2008; Hilhorst-Hofstee et al. 2011). 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 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.

P065/P066 specific notes:

- Mosaicism has been reported in individuals with Marfan syndrome. Mosaic *FBN1* cases obtained with the P065-C1/P066-C1 Marfan Syndrome probemixes must be confirmed by analysis of a second, independently collected DNA sample or a different technique.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *FBN1* and *TGFBR2* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemixes P065 Marfan Syndrome-1 and P066 Marfan Syndrome-2.
- 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.

***FBN1* and *TGFBR2* mutation database**

https://databases.lovd.nl/shared/genes/FBN1; https://databases.lovd.nl/shared/genes/TGFBR2; http://www.umd.be/FBN1/.

We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD) or the UMD-FBN1 mutation database. 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 *FBN1* exons 8 and 10 but not exon 9) to MRC Holland: info@mrcholland.com.

Table 1a. SALSA MLPA Probemix P065-C1 Marfan Syndrome-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*FBN1*	*TGFBR2*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L19287	5q		
136	**FBN1 probe** 17174-L20399		**Exon 62**	
142	**FBN1 probe** 04513-L14408		**Exon 2**	
149	Reference probe 10056-L10480	8q		
154	**FBN1 probe** 03931-L03386		**Exon 47**	
160	**FBN1 probe** 03922-L20371		**Exon 25**	
167	**FBN1 probe** 03914-L20834		**Exon 4**	
172	**TGFBR2 probe** 02797-L20835			**Exon 8**
178	**FBN1 probe** 17175-L20790		**Exon 52**	
184	**FBN1 probe** 03930-L03385		**Exon 43**	
190	**FBN1 probe** 03915-L03370		**Exon 7**	
196	**TGFBR2 probe** 03861-L03610			**Exon 3**
202	Reference probe 15424-L17583	7p		
208	**TGFBR2 probe** 17167-L21489			**Exon 4**
214	**FBN1 probe** 17168-L29998		**Exon 13**	
220	**FBN1 probe** 17179-L20404		**Exon 1**	
226	**FBN1 probe** 03924-L21131		**Exon 27**	
232	**FBN1 probe** 03932-L21132		**Exon 48**	
238	**FBN1 probe** 03925-L03380		**Exon 31**	
244	**FBN1 probe** 17182-L20407		**Exon 28**	
250	Reference probe 18915-L24510	1p		
256	**TGFBR2 probe** 03863-L03246			**Exon 5**
263	**FBN1 probe** 17184-L20409		**Exon 38**	
268	**FBN1 probe** 03933-L21562		**Exon 51**	
274	**FBN1 probe** 03917-L21563		**Exon 15**	
281	**FBN1 probe** 17186-L20411		**Exon 26**	
287	**FBN1 probe** 03926-L20793		**Exon 32**	
292	**TGFBR2 probe** 03864-L03247			**Exon 6**
299	**FBN1 probe** 21260-L29919		**Exon 21**	
305	**FBN1 probe** 21276-L29920		**Exon 64**	
314	**FBN1 probe** 21277-L29639		**Exon 54**	
319	**FBN1 probe** 03918-L03373		**Exon 16**	
328	**TGFBR2 probe** 03865-L03248			**Exon 7**
337	**FBN1 probe** 03927-L03382		**Exon 35**	
346	**FBN1 probe** 04337-L20895		**Exon 57**	
355	Reference probe 10134-L10596	18q		
363	**FBN1 probe** 17191-L20416		**Exon 49**	
373	**FBN1 probe** 03919-L03374		**Exon 19**	
382 ±	**TGFBR2 probe** 02795-L29999			**Exon 1**
391	**FBN1 probe** 03928-L03383		**Exon 37**	
400	**FBN1 probe** 17169-L20794		**Exon 63**	
408	**TGFBR2 probe** 04665-L29657			**Exon 1**
418	**FBN1 probe** 03920-L03375		**Exon 20**	
427	**FBN1 probe** 03929-L03750		**Exon 41**	
436	Reference probe 12790-L13925	2q		
445	Reference probe 09107-L09166	4q		
454	Reference probe 19329-L25556	7q		
463	**FBN1 probe** 03921-L20374		**Exon 22**	
471	**FBN1 probe** 17193-L20795		**Exon 60**	
480	**FBN1 probe** 17195-L21507		**Exon 11**	
493 ∫	**TGFBR2 probe** 17196-L20421			**Exon 2**
504	Reference probe 09870-L19465	2p		

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

± SNP rs138010137 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

∫ SNP rs184395862 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 1b. SALSA MLPA Probemix P066-C1 Marfan Syndrome-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*FBN1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L19287	5q	
136	Reference probe 20515-L28105	1q	
142	**FBN1 probe** 02447-L01891		**Exon 2**
148	**FBN1 probe** 21281-L29661		**Exon 36**
154	Reference probe 10694-L11276	6p	
160	**FBN1 probe** 02464-L01908		**Exon 39**
166	**FBN1 probe** 21278-L30097		**Exon 3**
172	**FBN1 probe** 02449-L01893		**Exon 5**
178	**FBN1 probe** 02465-L01909		**Exon 42**
184	**FBN1 probe** 02450-L01894		**Exon 6**
190	**FBN1 probe** 17176-L20401		**Exon 44**
196	Reference probe 19221-L25907	13q	
202	**FBN1 probe** 17177-L20402		**Exon 55**
208 Δ	**FBN1 probe** 17178-L20403		**Exon 65**
214	**FBN1 probe** 02467-L20809		**Exon 45**
220	**FBN1 probe** 02452-L01896		**Exon 8**
226	**FBN1 probe** 17180-L21453		**Exon 23**
232	**FBN1 probe** 02775-L26889		**Exon 61**
238	Reference probe 10089-L10513	8q	
244	**FBN1 probe** 17181-L20406		**Exon 59**
250	**FBN1 probe** 02453-L20807		**Exon 9**
256	**FBN1 probe** 02469-L01913		**Exon 50**
263	**FBN1 probe** 17183-L20408		**Exon 12**
269	**FBN1 probe** 02454-L20806		**Exon 10**
282	**FBN1 probe** 21259-L29867		**Exon 40**
286	Reference probe 12438-L13439	22q	
292	**FBN1 probe** 02773-L21454		**Exon 34**
299	**FBN1 probe** 21282-L29868		**Upstream**
310	**FBN1 probe** 02456-L01900		**Exon 14**
319	**FBN1 probe** 17528-L20415		**Exon 33**
328	Reference probe 21112-L29527	19p	
337	**FBN1 probe** 02457-L01901		**Exon 17**
346	**FBN1 probe** 02473-L01917		**Exon 58**
355 +	Reference probe 10293-L10805	2q	
364	**FBN1 probe** 19502-L26792		**Exon 66**
373	Reference probe 13280-L14613	1p	
382	**FBN1 probe** 02459-L01903		**Exon 24**
391	**FBN1 probe** 02774-L01912		**Exon 46**
400	**FBN1 probe** 02460-L01904		**Exon 29**
409	**FBN1 probe** 02476-L01920		**Exon 65**
418	Reference probe 19751-L26534	9q	
427	**FBN1 probe** 02461-L20803		**Exon 30**
436	**FBN1 probe** 02477-L01921		**Exon 66**
445	**FBN1 probe** 02772-L20802		**Exon 18**
454	Reference probe 15515-L17370	7q	
463	**FBN1 probe** 17192-L20417		**Exon 56**
472	Reference probe 18688-L14387	17q	
481	**FBN1 probe** 17194-L21455		**Exon 53**
490	Reference probe 14431-L21456	11p	

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

+ SNP rs201273354 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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. P065-C1/P066-C1 probes arranged according to chromosomal location

Table 2a. *FBN1*

Length (nt) P065	Length (nt) P066	SALSA MLPA probe	*FBN1* exon[a]	Ligation site[b] NM_ 000138.5	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*317-319 (Exon 2)*		
	299	21282-L29868	Upstream	1.0 kb before exon 1	CAGGCCTTCCTA-AACTGTTACAGG	1.0 kb
220		17179-L20404	Exon 1	9-10	GGCAGAGACTGT-GGGTGCCACAAG	1.0 kb
	142	02447-L01891	Exon 2	360-361	GGGATTTACCGT-GCTTTTAGCGTC	0.1 kb
142		04513-L14408	Exon 2	430-431	AAGGAAACCAGA-GCCAGTCGGGCC	31.6 kb
	166	21278-L30097	Exon 3	505-506	GGATCACGTTAT-AATGCTTACTGT	2.3 kb
167		03914-L20834	Exon 4	596-597	GGGATGGATTTT-GTTCGAGGCCAA	10.6 kb
	172	02449-L01893	Exon 5	688-689	ATTCGCTGTATG-AATGGAGGTAGC	3.8 kb
	184	02450-L01894	Exon 6	781-782	AGTGGCTGTCTC-AATGGAGGAAGG	58.7 kb
190		03915-L03370	Exon 7	1015-1016	CCCTGCCGCCGT-GGCTTCATTCCA	3.6 kb
	220	02452-L01896	Exon 8	1170-1171	AGTGTCACAAAA-ATGTGAAGGTAA	7.9 kb
	250	02453-L20807	Exon 9	1237-1238	TGTACAAACACA-GTCAGCAGTTAC	5.5 kb
	269	02454-L20806	Exon 10	1432-1433	GGGGTCACTGTC-GCCCCTGAGATG	4.4 kb
480		17195-L21507	Exon 11	1489-1490	CTGTGCTCTGTT-CCTATGGTAATT	0.9 kb
	263	17183-L20408	Exon 12	1771-1772	CTGGACCTCCGT-GGGGAGTGTATT	1.8 kb
214		17168-L29998	Exon 13	1831-1832	GGTGAGTGTATT-AACAACCAGGGT	3.5 kb
	310	02456-L01900	Exon 14	1990-1991	TGCGTGTGTAAT-GCGGGCTTTCAT	1.5 kb
274		03917-L21563	Exon 15	2118-2119	TTGCAAACCTGG-ATTCCAGCTGGC	3.5 kb
319		03918-L03373	Exon 16	2228-2229	CCTACAGATGTG-AATGCTTCCCTG	1.3 kb
	337	02457-L01901	Exon 17	2402-2403	TTGGGGAACCTT-GCCAGCCGTGTC	4.8 kb
	445	02772-L20802	Exon 18	2453-2454	CACTCTGCAGCA-GTGGGCCAGGAA	1.7 kb
373		03919-L03374	Exon 19	2524-2525	ATTTGCCCAAAT-GGAATCTGTGAA	1.2 kb
418		03920-L03375	Exon 20	2661-2662	TGGACAATGTAG-AAATACTCCTGG	0.6 kb
299		21260-L29919	Exon 21	2741-2740; reverse	TTCGCATTCATC-AATGTCTGAAAC	0.4 kb
463		03921-L20374	Exon 22	2884-2885	TGCTGGCAGACT-GTCATTGATGGG	1.0 kb
	226	17180-L21453	Exon 23	3024-3025	GTACTCAAGAAT-TAAAGGAACACA	1.7 kb
	382	02459-L01903	Exon 24	3133-3134	CAGTGTCCCAGT-GGAATGACTTTG	2.6 kb
160		03922-L20371	Exon 25	3316-3317	CCCATGAGAAAT-ACTCCTGAGTAC	1.6 kb
281		17186-L20411	Exon 26	27 nt after exon 26	TTAAGGGCCAGG-AGAGGGGACGTC	0.1 kb
226		03924-L21131	Exon 27	3559-3560	CCTGACCTCTGT-GGCAGAGGCCAG	0.8 kb
244		17182-L20407	Exon 28	3691-3692	CTCCTATGCCGA-GGTGGTGTTTGC	0.3 kb
	400	02460-L01904	Exon 29	3860-3861	ATCAGTGTGCCT-GCAACCCTGGCT	1.7 kb
	427	02461-L20803	Exon 30	3987-3988	ATGTAGCTGTCA-GCCGGGATTTGC	1.5 kb
238		03925-L03380	Exon 31	4090-4091	ACAAATATCCCT-GGAGAGTACAGG	2.2 kb
287		03926-L20793	Exon 32	4213-4214	TGTGAAAACACG-AAAGGCTCATTT	7.2 kb
	319	17528-L20415	Exon 33	4372-4373	AGCTGCAGTCCC-GGGTGGATTGGA	0.3 kb
	292	02773-L21454	Exon 34	4481-4482	GATCTTACCGCT-GTCTGTGCAAGG	1.7 kb
337		03927-L03382	Exon 35	4567-4568	CTCTGTGGCAAT-GGCCAGTGCCTC	2.0 kb

Length (nt) P065 P066	SALSA MLPA probe	*FBN1* exon[a]	Ligation site[b] NM_ 000138.5	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
148	21281-L29661	Exon 36	5 nt after exon 36; reverse	GCCAGTGGAGGT-CTTACCTGTGCA	2.1 kb
391	03928-L03383	Exon 37	4811-4812	CCACGTGCATCA-GTGGGAACTGTG	0.5 kb
263	17184-L20409	Exon 38	4966-4967	ACAGCCTGCAGC-AATGAAATTGGA	2.2 kb
160	02464-L01908	Exon 39	5089-5090	CTTTGTCCTGGA-GGGGAAGGTTTC	0.3 kb
282	21259-L29867	Exon 40	5255-5256	CACGAGTGTGTG-ATGGTAAATGGT	1.6 kb
427	03929-L03750	Exon 41	5290-5291	CCTGGAATCTGT-GGTCCAGGGACA	0.8 kb
178	02465-L01909	Exon 42	5450-5451	GAGAATTGTTAT-TCAACATGACCA	2.9 kb
184	03930-L03385	Exon 43	5599-5600	GACATTTATACC-GGTTTACCCGTT	3.5 kb
190	17176-L20401	Exon 44	5 nt before exon 44	GACTACTCTGTC-TCTAGATATTGA	4.2 kb
214	02467-L20809	Exon 45	5816-5817	GCTACCGCTGTG-ACTGTAAGCCCG	3.8 kb
391	02774-L01912	Exon 46	5904-5905	ATGCAGTCATGG-GCAGTGCATTGA	2.1 kb
154	03931-L03386	Exon 47	6024-6025	CTGTGGGAATGG-AACTTGCCGGAA	1.4 kb
232	03932-L21132	Exon 48	6188-6189	TCCAGTGCCAGT-GCAATGAAGGCT	0.8 kb
363	17191-L20416	Exon 49	6320-6321	GCATTTGCCCAC-CTGGATACAGTC	2.8 kb
256	02469-L01913	Exon 50	6419-6420	ACACTGAAGGCA-GCTTCAAATGTC	4.0 kb
268	03933-L21562	Exon 51	6596-6597	AAGGCTGGGGAG-ACCCCTGCGAGC	0.4 kb
178	17175-L20790	Exon 52	18 nt before exon 52	TCTATCTATTAA-TGAGTGTCTCCA	0.5 kb
481	17194-L21455	Exon 53	6812-6813	ATGAATGTGTAG-GTGAGTAATAAG	2.3 kb
314	21277-L29639	Exon 54	6885-6886	AGGTTTTGAATG-CACCTGCGAGGA	1.8 kb
202	17177-L20402	Exon 55	6 nt after exon 55	GCAAAGGTGAGT-CATCGTGTTCAA	2.2 kb
463	17192-L20417	Exon 56	15 nt after exon 56	AGAGGATCCCTG-TGGAAGGAGCTT	2.3 kb
346	04337-L20895	Exon 57	7262-7263	GGAGCTACACCT-GTGAGTGTAATG	0.7 kb
346	02473-L01917	Exon 58	7347-7348	CACAGAGGTGCT-ACAAAACATGTG	2.0 kb
244	17181-L20406	Exon 59	7593-7594	AGGATCATATCA-TTGCATTTGTAA	0.4 kb
471	17193-L20795	Exon 60	7731-7732	TTCATGCCCGAA-AGGCTACATTCT	3.4 kb
232	02775-L26889	Exon 61	7847-7848	TCACATGCAAAT-GTCCTCCCGGAT	0.3 kb
136	17174-L20399	Exon 62	26 nt before exon 62	GAATTTTAACCC-CTCTTTGCCCCC	0.9 kb
400	17169-L20794	Exon 63	8042-8043	AGGGTAACCACC-GCTGCCAGCATG	5.2 kb
305	21276-L29920	Exon 64	8324-8325	CCGAGGGCGGTT-ACCTGTGTGGCT	2.9 kb
208 Δ	17178-L20403	Exon 65	8397-8398	GGGCATGGGCCG-AGGAAACCCAGA	0.1 kb
409	02476-L01920	Exon 65	8527-8528	AACGAAACTGAT-GCCTCCAATATC	1.2 kb
436	02477-L01921	Exon 66	8551-8552	CAGGATCAGTCT-GAGACAGAAGCC	0.8 kb
364	19502-L26792	Exon 66	9333-9334	TAGGTTGTCCAT-TTATGGTACCTA	
		stop codon	*8930-8932 (Exon 66)*		

Table 2b. *TGFBR2*

Length (nt) P065	SALSA MLPA probe	*TGFBR2* exon[a]	Ligation site[b] NM_001024847.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*284-286 (Exon 1)*		
382 ±	02795-L29999	Exon 1	9 nt before exon 1	TGAGTTGAAGTT-GAGTGAGTCACT	0.2 kb
408	04665-L29657	Exon 1	230-231	GGGGTCCGGGAA-GGCGCCGTCCGC	16.4 kb
493 ∫	17196-L20421	Exon 2	4 nt after exon 2	GAGACATAGTAA-AGTATCATTAAT	21.6 kb
196	03861-L03610	Exon 3	549-550	CTGTGACAACCA-GAAATCCTGCAT	5.5 kb
208	17167-L21489	Exon 4	672-673	CCATGACCCCAA-GCTCCCCTACCA	21.4 kb
256	03863-L03246	Exon 5	897-898	GGGAGTTGCCAT-ATCTGTCATCAT	2.5 kb
292	03864-L03247	Exon 6	1707-1708	TGTCTACTCCAT-GGCTCTGGTGCT	14.3 kb
328	03865-L03248	Exon 7	1830-1831	GAAGGACAACGT-GTTGAGAGATCG	3.0 kb
172	02797-L20835	Exon 8	1908-1909	GTGTGAGACGTT-GACTGAGTGCTG	
		stop codon	*2060-2062 (Exon 8)*		

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

[b] For some probes, the ligation site is located outside the exon, as it was not possible to design a specific probe with a ligation site within the exon.

[c] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNP rs138010137 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

∫ SNP rs184395862 influences the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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.

Related SALSA MLPA probemixes

P148 TGFBR1-TGFBR2	Contains probes for *TGFBR1* and *TGFBR2*, involved in aortic aneurysm syndrome. The majority of the *TGFBR2* probes in P148 have the same ligation site as the *TGFBR2* probes in P065.

References

- Baetens M et al. (2011). Applying massive parallel sequencing to molecular diagnosis of Marfan and Loeys-Dietz syndromes. *Hum Mutat.* 32:1053-1062.
- Blyth M et al. (2008). Severe Marfan syndrome due to FBN1 exon deletions. *Am J Med Genet A.* 146A:1320-1324.
- Campbell IM et al. (2011). TGFBR2 deletion in a 20-month-old female with developmental delay and microcephaly. *Am J Med Genet A.* 155A:1442-1447.
- Dietz HC et al. (1991). Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature.* 352:337-339.
- Hilhorst-Hofstee Y et al. (2011). The clinical spectrum of complete FBN1 allele deletions. *Eur J Hum Genet.* 19:247-252.
- Hung CC et al. (2009). Mutation spectrum of the fibrillin-1 (FBN1) gene in Taiwanese patients with Marfan syndrome. *Ann Hum Genet.* 73:559-567.
- Lee B et al. (1991). Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. *Nature.* 352:330-334.
- Lerner-Ellis JP et al. (2014). The spectrum of FBN1, TGFbetaR1, TGFbetaR2 and ACTA2 variants in 594 individuals with suspected Marfan Syndrome, Loeys-Dietz Syndrome or Thoracic Aortic Aneurysms and Dissections (TAAD). *Mol Genet Metab.* 112:171-176.
- Mannucci L et al. (2020). Mutation analysis of the FBN1 gene in a cohort of patients with Marfan Syndrome: A 10-year single center experience. *Clin Chim Acta.* 501:154-164.
- Rand-Hendriksen S et al. (2007). Search for correlations between FBN1 genotype and complete Ghent phenotype in 44 unrelated Norwegian patients with Marfan syndrome. *Am J Med Genet A.* 143A:1968-1977.
- 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.
- Stengl R et al. (2020). Optimising the mutation screening strategy in Marfan syndrome and identifying genotypes with more severe aortic involvement. *Orphanet J Rare Dis.* 15:290.
- 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 Probemixes P065/P066 Marfan Syndrome

- Benke K et al. (2018). Hungarian Marfan family with large FBN1 deletion calls attention to copy number variation detection in the current NGS era. *J Thorac Dis.* 10:2456-2460.
- Dordoni C et al. (2017). Marfan syndrome: Report of a complex phenotype due to a 15q21.1 contiguos gene deletion encompassing FBN1, and literature review. *Am J Med Genet A.* 173:200-206.
- Franken R et al. (2016). Genotype impacts survival in Marfan syndrome. *Eur Heart J.* 37:3285-3290.
- Franken R et al. (2017). Relationship between fibrillin-1 genotype and severity of cardiovascular involvement in Marfan syndrome. *Heart.* 103:1795-1799.
- 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.
- Li J et al. (2017). Gross deletions in FBN1 results in variable phenotypes of Marfan syndrome. *Clin Chim Acta.* 474:54-59.
- Matyas G et al. (2007). Large genomic fibrillin-1 (FBN1) gene deletions provide evidence for true haploinsufficiency in Marfan syndrome. *Hum Genet.* 122:23-32.
- 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.
- Nazarali S et al. (2017). Childhood glaucoma in neonatal Marfan syndrome resulting from a novel FBN1 deletion. *Can J Ophthalmol.* 52:e171-e173.
- Ogawa N et al. (2011). Evaluating Japanese patients with the Marfan syndrome using high-throughput microarray-based mutational analysis of fibrillin-1 gene. *Am J Cardiol.* 108:1801-1807.
- Pees C et al. (2014). Detection of 15 novel mutations in 52 children from 40 families with the Marfan or Loeys-Dietz syndrome and phenotype-genotype correlations. *Clin Genet.* 86:552-557.
- Proost D et al. (2015). Performant Mutation Identification Using Targeted Next-Generation Sequencing of 14 Thoracic Aortic Aneurysm Genes. *Hum Mutat.* 36:808-814.
- Wojcik MH et al. (2019). Genome Sequencing Identifies the Pathogenic Variant Missed by Prior Testing in an Infant with Marfan Syndrome. *J Pediatr.* 213:235-240.
- Wooderchak-Donahue W et al. (2015). Clinical utility of a next generation sequencing panel assay for Marfan and Marfan-like syndromes featuring aortopathy. *Am J Med Genet A.* 167A:1747-1757.
- Yang H et al. (2018). Identification of gross deletions in FBN1 gene by MLPA. *Hum Genomics.* 12:46.

P065 product history	
Version	*Modification*
C1	One *FBN1* target probe and three reference probes have been replaced. Several probes have been modified in length, but not in the sequence detected.
B1	One new *TGFBR2* probe and seven new *FBN1* probes have been added, three *FBN1* probes have been replaced, all reference probes have been replaced and new QDX2 control fragments have been added.
A2	Two reference probes have been replaced and X- and Y- specific control fragments have been added.
A1	First release.

P066 product history	
Version	*Modification*
C1	Two *FBN1* target probes have been replaced and one *FBN1* promoter probe has been added. Also, five reference probes have been replaced.
B2	One reference probe has been removed and two probes have been adjusted.
B1	Four new *FBN1* probes have been added, eight *FBN1* probes have been replaced, and all reference probes have been replaced.
A3	The 88 and 96 nt control fragments have been replaced (QDX2).
A2	DNA control fragments (D-fragments) and X- and Y- specific control fragments have been added.
A1	First release.

Implemented changes in the product description
Version C1/C1-04 – 20 December 2023 (04P) - Warning about variability of 208 nt probe 17178-L20403 (P066) added in Table 1b and Table 2a. - Remark on mosaicism added to P065/P066 specific notes. - Section selected publications using SALSA MLPA Probemixes P065/P066 Marfan Syndrome adjusted. - Ligation sites of the probes targeting the *TGFBR2* gene updated according to new version of the NM_ reference sequence. - Morocco is removed from the list of countries where the SALSA MLPA Probemixes P065/P066 Marfan Syndrome are CE-marked. Version C1/C1-03 – 26 April 2021 (04P) - Product description rewritten and adapted to a new template. - Intended purpose updated. - Clinical background and performance characteristics updated. - Two additional positive control DNA samples added. - Links to mutation databases updated. - List of selected publications using SALSA MLPA probemixes P065/P066 Marfan Syndrome updated. - UK added to the list of countries in Europe that accept the CE mark. Version C1/C1-02 — 10 January 2020 (02P) - Product description rewritten and adapted to a new template. - Intended use updated. - Morocco and Israel added as countries with IVD status. - Ligation sites of the probes targeting the *FBN1* gene updated according to the new version of the NM_ reference sequence. - Warning about off-scale peaks added to the interpretation of results section. - Small changes of probe lengths in Table 1a in order to better reflect the true lengths of the amplification products. - Remark about a SNP influencing the probe signal added to the following probes: 382 nt probe 02795-L29999 (P065), 493 nt probe 17196-L20421 (P065), and 355 nt probe 10293-L10805 (P066). - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). - List with selected publications using SALSA MLPA probemixes P065/P066 Marfan Syndrome updated.

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