Product Description
SALSA® MLPA® Probemix P285-C3 LRP5

To be used with the MLPA General Protocol.

Version C3
For complete product history see page 8.

Catalogue numbers:
- **P285-025R:** SALSA MLPA Probemix P285 LRP5, 25 reactions.
- **P285-050R:** SALSA MLPA Probemix P285 LRP5, 50 reactions.
- **P285-100R:** SALSA MLPA Probemix P285 LRP5, 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 P285 LRP5 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *LRP5*, *DKK1*, *NDP* and *FZD4* genes, which are associated with osteoporosis-pseudoglioma syndrome (OPPG / OPPS) and exudative vitreoretinopathy (EVR). This probemix can also be used to detect the presence of the *LRP5* G171V point mutation.

OPPG or OPPS and EVR are caused by defects in the same genes: *LRP5*, *NDP* and *FZD4* genes, amongst others. Gong et al. (2001) demonstrated in situ low density lipoprotein receptor (LRP5) expression by osteoblasts, which showed that LRP5 can transduce Wnt signalling in vitro via the canonical pathway. They further showed that a mutant secreted form of LRP5 could reduce bone thickness in mouse calvarial explant cultures. These data indicated that Wnt-mediated signalling via LRP5 affects bone accrual during growth and is important for the establishment of peak bone mass. *LRP5* deletions result in decreased bone mass. Gain of function *LRP5* mutations resulting in increased bone density have also been described. A probe for one gain of function mutation, G171V, is included in this probemix. Dickkopf-1 (DKK1) is a soluble inhibitor of Wnt/beta-catenin signalling required for embryonic head development. DKK1 regulates Wnt signalling by binding to the Wnt co-receptor LRP5. Mice heterozygous for *DKK1* deletion display an increased bone formation and a high bone mass phenotype. Defects in *NDP* have been reported in Norrie disease and in X-linked exudative vitreoretinopathy. Finally, heterozygous mutations in the frizzled-4 gene (*FZD4*), which encodes 7-transmembrane domain proteins that are receptors for Wnt signaling proteins, are also reported to cause familial exudative vitreoretinopathy-1 (EVR1).

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 *LRP5*, *DKK1*, *NDP* and *FZD4* exon numbering used in this P285-C3 LRP5 product description is the exon numbering from the NG_015835.2, NM_012242.4, NG_009832.1, and NG_011752.1 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 P285-C3 LRP5 contains 46 MLPA probes with amplification products between 128 and 481 nucleotides (nt). This includes 24 probes for *LRP5* gene, four probes for *DKK1* gene, three probes for *FZD4* gene, and three probes for *NDP* gene. Furthermore, this probemix also contains one probe specific for the G171V mutation located in exon 3 of the *LRP5* gene, which will only generate a signal when the mutation is present. In addition, 11 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 osteoporosis-pseudoglioma syndrome and exudative vitreoretinopathy. It is recommended 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 samples should be validated before use.

SALSA Binning DNA SD030

The SD030 Binning DNA provided with this probemix can be used for binning of all probes including the mutation-specific probe (202 nt probe 09270-SP0044-L09500 G171V mutation). SD030 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD030 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD030 Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

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 when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	Duplication	1.75 < FR < 2.15
Ambiguous copy number		All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs,

point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *LRP5* and *FZD4* genes. 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 *LRP5*, *DKK1*, *NDP* and *FZD4* genes are small (point) mutations, next to the *LRP5* G171V point mutation, none of which will not be detected by using SALSA MLPA Probemix P285 LRP5.
- 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.

Malacards mutation database

https://www.malacards.org/card/osteoporosis_pseudoglioma_syndrome. We strongly encourage users to deposit positive results in the Malacards Human Disease 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 *LRP5* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P285-C3 LRP5

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*LRP5*	*DKK1*	*NDP*	*FZD4*
64-105	Control fragments – see table in probemix content section for more information					
128	Reference probe 19246-L25351	16q				
136	**LRP5 probe** 09285-L09516		**Exon 16**			
142 «	**LRP5 probe** 09291-L09522		**Exon 22**			
149	Reference probe 07023-L21989	14q				
160	**DKK1 probe** 09267-L09527			**Exon 4**		
166 «	**FZD4 probe** 13204-L14525				**Exon 1**	
172	**LRP5 probe** 09287-L26131		**Exon 18**			
178	**LRP5 probe** 09283-L30495		**Exon 14**			
184	**LRP5 probe** 09286-L09517		**Exon 17**			
190	**LRP5 probe** 09277-L09507		**Exon 9**			
196	Reference probe 12062-L04183	4q				
202 § Ж	**LRP5 probe** 09270-SP0044-L09500		**Exon 3**			
208 «	**LRP5 probe** 12379-L13725		**Exon 1**			
215	**LRP5 probe** 10338-L30305		**Exon 19**			
220	Reference probe 12427-L13428	22q				
228	**LRP5 probe** 09284-L10875		**Exon 15**			
235	Reference probe 08056-L30306	5p				
240 «	**LRP5 probe** 09292-L30307		**Exon 23**			
245 «	**LRP5 probe** 19767-L30308		**Exon 3**			
252	**LRP5 probe** 14488-L30309		**Exon 7**			
258	**DKK1 probe** 09266-L09526			**Exon 3**		
264	**DKK1 probe** 09265-L10877			**Exon 2**		
274 Δ	**LRP5 probe** 10341-L09506		**Exon 8**			
281	Reference probe 16919-L19863	7p				
292	**LRP5 probe** 09282-L09513		**Exon 13**			
299 «	**LRP5 probe** 09273-L09503		**Exon 5**			
310	**LRP5 probe** 11604-L11383		**Exon 20**			
320	Reference probe 06602-L06160	8q				
328	**LRP5 probe** 09281-L09512		**Exon 12**			
337 «	**FZD4 probe** 13205-L14526				**Exon 2**	
346 «	**LRP5 probe** 14485-L16205		**Exon 4**			
353 «	**LRP5 probe** 13206-L14527		**Exon 1**			
361	Reference probe 08674-L08686	9q				
370	**DKK1 probe** 09264-L09524			**Exon 1**		
382	**LRP5 probe** 09269-L09498		**Exon 2**			
391	Reference probe 07234-L06884	1q				
400 «	**LRP5 probe** 10343-L09521		**Exon 21**			
410	**LRP5 probe** 09280-L09511		**Exon 11**			
418	**LRP5 probe** 14487-L16207		**Exon 6**			
426	**NDP probe** 13207-L14528					**Exon 3**
436	Reference probe 06483-L06009	1p				
445	**NDP probe** 13208-L14529					**Exon 1**
454 «	**FZD4 probe** 13209-L14530				**Exon 1**	
463	**NDP probe** 13210-L14531					**Exon 2**
472	**LRP5 probe** 21482-L20422		**Exon 10**			
481	Reference probe 09966-L10425	17q				

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

§ Mutation-specific probe. This probe will only generate a signal when the G171V mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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. P285-C3 probes arranged according to chromosomal location

Table 2a. *DKK1*

Length (nt)	SALSA MLPA probe	*DKK1* exon[a]	Ligation site NM_012242.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*155-157 (Exon 1)*		
370	09264-L09524	Exon 1	266-267	ACTCGGTTCTCA-ATTCCAACGCTA	0.5 kb
264	09265-L10877	Exon 2	12 nt after exon 2	GTGAGTCCTGAA-AGCTCCCTTTCA	1.2 kb
258	09266-L09526	Exon 3	601-602	TTCCGAGGAGAA-ATTGAGGAAACC	0.4 kb
160	09267-L09527	Exon 4	900-901	CCGGATACAGAA-AGATCACCATCA	
		stop codon	*953-955 (Exon 4)*		

Table 2b. *LRP5*

Length (nt)	SALSA MLPA probe	*LRP5* exon[a]	Ligation site NM_002335.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*125-127 (Exon 1)*		
353 «	13206-L14527	Exon 1	104 nt after exon 1	CGGAAGCGACTT-GGCGAGTTGGGA	0.4 kb
208 « #	12379-L13725	Exon 1	454 nt after exon 1	GGACGGGCTGAA-GTTGCAGCGAGA	34.7 kb
382	09269-L09498	Exon 2	339-340	GGACTTCCAGTT-TTCCAAGGGAGC	9.7 kb
202 § Ж	09270-SP0044-L09500	Exon 3	636-637; 661-662	GACAGACTGGGT-25 nt spanning oligo-GGGATGGATGGC	0.1 kb
245 « #	19767-L30308	Exon 3	743-744	ACCTGGAGGAGC-AGAAGCTCTACT	6.0 kb
346 «	14485-L16205	Exon 4	883-884	CTGTACTGGACA-GACTGGCAGACC	1.8 kb
299 «	09273-L09503	Exon 5	1069-1070	CTGTCCCCAAGC-GAGCCTTTCTAC	20.8 kb
418	14487-L16207	Exon 6	1294-1295	GTCTACTGGACA-GATGACGAGGTG	3.6 kb
252	14488-L30309	Exon 7	18 nt after exon 7	CTCCTGTGGACA-TGTTTGATCCAG	13.4 kb
274 Δ	10341-L09506	Exon 8	1716-1717	GCAGGTGATCAA-TGTTGATGGGAC	3.3 kb
190 #	09277-L09507	Exon 9	2158-2159	AAGGAGGCCTCA-GCCCTGGACTTT	3.2 kb
472	21482-L20422	Exon 10	2268-2269	CGTGGTGGAGTT-TGGCCTTGACTA	1.6 kb
410	09280-L09511	Exon 11	2604-2605	GGACACCAACAT-GATCGAGTCGTC	2.2 kb
328	09281-L09512	Exon 12	2714-2715	GGACAGACTGGA-ATCTGCACAGCA	2.7 kb
292	09282-L09513	Exon 13	3076-3077	GCCATCGACTAT-GACCCACTGGAC	7.1 kb
178	09283-L30495	Exon 14	3184-3185	AGCCAAGGCCAA-AACCCAGACAGG	1.8 kb
228	09284-L10875	Exon 15	3531-3532	CCTGAAGCGCAT-TGAGAGCTGTGA	0.8 kb
136	09285-L09516	Exon 16	3626-3627	GCAAGCATCTCT-ACTGGATCGACC	3.6 kb
184	09286-L09517	Exon 17	3810-3811	CCACATCTGTAT-TGCCAAGGGTGA	4.2 kb
172	09287-L26131	Exon 18	4093-4094	GAGGCAGACTGT-CAGGACCGCTCA	3.1 kb
215	10338-L30305	Exon 19	4179-4180	TGTCCTCATCAA-ACAGCAGTGCGA	1.6 kb
310	11604-L11383	Exon 20	4337-4338	GTGGTGTCTATT-TTGTGTGCCAGC	1.3 kb
400 «	10343-L09521	Exon 21	4582-4583	TCCAGCAGCTCG-TCCAGCACGAAG	6.5 kb
142 «	09291-L09522	Exon 22	12 nt before exon 22	TCACCCTGGCTT-GGCTCTCCTCAG	2.5 kb
240 «	09292-L30307	Exon 23	4820-4821	ACTACCTGGATT-TGAACTCGGACT	
		stop codon	*4970-4972 (Exon 23)*		

Table 2c. *FZD4*

Length (nt)	SALSA MLPA probe	*FZD4* exon[a]	Ligation site NM_012193.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*311-313 (Exon 1)*		
454 «	13209-L14530	Exon 1	382-383	CTGGGGTTGCTC-CTGCAGTTGCTG	0.2 kb
166 «	13204-L14525	Exon 1	553-554	CAGCTGACAACT-TTCACACCGCTC	2.5 kb
337 «	13205-L14526	Exon 2	712-713	GTCCTGAAGGAA-TTTGGATTTGCC	
		stop codon	*1922-1924 (Exon 2)*		

Table 2d. *NDP*

Length (nt)	SALSA MLPA probe	*NDP* exon[a]	Ligation site NM_000266.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*295-297 (Exon 2)*		
445	13208-L14529	Exon 1	10-11	ACAGAAGAACAA-AAGCATTTGGAA	14.8 kb
463	13210-L14531	Exon 2	392-393	ATTCATAATGGA-CTCGGACCCTCG	8.6 kb
426	13207-L14528	Exon 3	550-551	GCACTGTCCTCA-AGCAACCCTTCC	
		stop codon	*694-696 (Exon 3)*		

[a] See section Exon numbering on page 2 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.

§ Mutation-specific probe. This probe will only generate a signal when the G171V mutation is present. It has been tested on artificial DNA **but not on positive human samples!**
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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.

Complete probe sequences are available at www.mrcholland.com.

References

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Selected publications using SALSA MLPA Probemix P285 LRP5

- Arai E et al. (2014). Familial cases of Norrie disease detected by copy number analysis. *Jpn J Ophthalmol*, 58(5), 448-454.
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- Rodríguez-Muñoz A et al. (2018). The importance of biochemical and genetic findings in the diagnosis of atypical Norrie disease. *Clin Chem Lab Med* (CCLM), 56(2), 229-235.
- Seo SH et al. (2015). Molecular characterization of FZD4, LRP5, and TSPAN12 in familial exudative vitreoretinopathy. *Invest Ophthalmol Vis Sci*, 56(9), 5143-5151.
- Seo SH et al. (2016). Large deletions of TSPAN12 cause familial exudative vitreoretinopathy (FEVR). *Invest Ophthalmol Vis Sci*, 57(15), 6902-6908.

P285 product history	
Version	*Modification*
C3	One reference probe has been added and two have been replaced. In addition, several lengths have been adjusted.
C2	Four reference probes have been replaced and the control fragments have been adjusted (QDX2).
C1	Exons 4, 6, and 7 have been added.
B1	Several probes have been added.
A1	First release.

Implemented changes in the product description

Version C3-01 – 05 January 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *LRP5*, *FZD4*, and *NDP* genes updated according to new version of the NM_ reference sequence.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Warning added for the LRP5 exon probe on 274 nt, this probe may be sensitive to certain experimental conditions.
- Warnings added for several probes for *LRP5* and *FZD4* genes, these probes are more sensitive to salt contamination.

Version 07 – 13 November 2017 (55)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and 2, new picture included).
- Ligation sites checked and if needed adjusted according NCBI RefSeq.
- Changes of probe lengths in Table 1 and Table 2 in order to better reflect the true lengths of the amplification products.
- Various textual and layout changes throughout the document.

Version 06 (53)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).

Version 05 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 04 (48)
- Warning about salt sensitivity of 238 nt probe 09292-L09523 and SNPs in target sequence of 436 nt probe 07596-L07281 added to Table 1.
- Remark on RefSeqGene standard and transcript variant added below Table 2.
- Various minor textual changes.
- Various minor layout changes.

Version 03 (45)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Table 2 for more than one gene/region has been named Table 2a - 2d.
- Sentence “when only small numbers of samples are tested, visual comparison of peak profiles should be sufficient” removed from data analysis section
- Ligation sites updated according to new version of the NM_reference sequences.

- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various minor textual changes on page 1, various minor layout changes.

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