Product Description SALSA® MLPA® Probemix P271-B5 COL1A1

To be used with the MLPA General Protocol.

Version B5. Compared to version B4, one reference probe has been replaced, one reference probe has been removed, and one probe length has been adjusted. For complete product history see page 7.

Catalogue numbers:
- **P271-025R:** SALSA MLPA Probemix P271 COL1A1, 25 reactions.
- **P271-050R:** SALSA MLPA Probemix P271 COL1A1, 50 reactions.
- **P271-100R:** SALSA MLPA Probemix P271 COL1A1, 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.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 P271 COL1A1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *COL1A1* gene, which is associated with osteogenesis imperfecta (OI).

OI is a genetic disorder characterized by bone fragility, severe bowing of long bones, and low bone mass. The prevalence of this disorder ranges from one per 10,000 to one per 20,000 live births. Severe forms of OI lead to intrauterine fractures and perinatal lethality. Besides bone, other tissues rich in type I collagen are also affected, including skin, ligaments, and tendons. The majority of patients diagnosed with OI have mutations in the *COL1A1* or *COL1A2* genes.

Type I collagen is the most common type of collagen and it is present in almost all connective tissues. This protein consists of three polypeptide chains: two α_1 polypeptide chains and one α_2 polypeptide chain. The α_1 polypeptide is encoded by the *COL1A1* gene and the α_2 polypeptide by the *COL1A2* gene.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1295/.

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 *COL1A1* exon numbering used in this P271-B5 COL1A1 product description is the exon numbering from the RefSeq transcript NM_000088.3, which is identical to the LRG_1 sequence. The exon numbering and NM_ sequence used have been retrieved on 03/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P271-B5 COL1A1 contains 42 MLPA probes with amplification products between 130 and 445 nucleotides (nt). This includes 33 probes for the *COL1A1* gene targeting 33 out of 51 exons of the gene. In addition, nine 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.mlpa.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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment 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.mlpa.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 unrelated individuals who are from families without a history of osteogenesis imperfecta. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.mlpa.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 dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *COL1A1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P271 COL1A1.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

COL1A1 **mutation database:** https://databases.lovd.nl/shared/genes/COL1A1. 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 SNPs and unusual results (e.g., a duplication of *COL1A1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P271-B5 COL1A1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*COL1A1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136	**COL1A1 probe** 13715-L15197		**Exon 3**
142	**COL1A1 probe** 07968-L07957		**Exon 30**
148	**COL1A1 probe** 07956-L07738		**Exon 9**
154	**COL1A1 probe** 07972-L07753		**Exon 36**
160	**COL1A1 probe** 07961-L07743		**Exon 18**
166	**COL1A1 probe** 07976-L07757		**Exon 43**
172	**COL1A1 probe** 07965-L07747		**Exon 25**
178	**COL1A1 probe** 07981-L29327		**Exon 49**
184	Reference probe 02312-L01803	19p13	
190 ±	**COL1A1 probe** 07953-L07735		**Exon 5**
196	Reference probe 04985-L23260	8q13	
202	**COL1A1 probe** 07955-L07737		**Exon 7**
208	Reference probe 04587-L03792	3q26	
215	**COL1A1 probe** 07973-L09423		**Exon 38**
221	**COL1A1 probe** 07957-L07739		**Exon 11**
229	**COL1A1 probe** 07974-L07755		**Exon 40**
238	**COL1A1 probe** 07960-L07742		**Exon 16**
247	**COL1A1 probe** 07978-L07759		**Exon 46**
256	Reference probe 03993-L03260	5q14	
263	**COL1A1 probe** 07963-L07745		**Exon 21**
274	**COL1A1 probe** 12246-L13183		**Exon 48**
283	**COL1A1 probe** 07967-L07749		**Exon 29**
292	**COL1A1 probe** 07951-L07733		**Exon 2**
299	Reference probe 12812-L19220	11p15	
306	**COL1A1 probe** 07977-L14627		**Exon 45**
312	**COL1A1 probe** 07959-L09424		**Exon 14**
322	**COL1A1 probe** 07979-L14628		**Exon 47**
328	**COL1A1 probe** 07962-L07744		**Exon 20**
337	Reference probe 20129-L27526	9q21	
346	**COL1A1 probe** 07982-L07763		**Exon 50**
355	**COL1A1 probe** 07966-L09425		**Exon 27**
363	**COL1A1 probe** 07983-L07764		**Exon 51**
377 *	Reference probe 19135-L29175	21q21	
390	**COL1A1 probe** 12247-L22282		**Exon 1**
398	**COL1A1 probe** 07964-L07746		**Exon 23**
405	**COL1A1 probe** 07954-L09429		**Exon 6**
411 ¥	**COL1A1 probe** 21675-L32137		**Exon 32**
419	**COL1A1 probe** 07971-L14814		**Exon 34**
427	**COL1A1 probe** 12248-L14813		**Exon 12**
436	**COL1A1 probe** 07975-L07756		**Exon 42**
445	Reference probe 06058-L05513	4p16	

a) See above section on exon numbering for more information.

* New in version B5.
¥ Changed in version B5. Minor alteration, no change in sequence detected.
± SNP rs72667018 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 2. *COL1A1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*COL1A1* exon[a]	Ligation site NM_000088.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*127-129 (Exon 1)*		
390	12247-L22282	Exon 1	31 nt after exon 1	GAGTGCAAGGAT-ACTCTATATCGC	1.5 kb
292	07951-L07733	Exon 2	265-266	ACGGCCTCAGGT-ACCATGACCGAG	0.3 kb
136	13715-L15197	Exon 3	448-449	ACCAAGAAACCA-CCGGCGTCGAGG	0.3 kb
	No probe	Exon 4			
190 ±	07953-L07735	Exon 5	4 nt after exon 5, reverse	GGCCTCTCCACT-TACTCCTCCGAG	0.8 kb
405	07954-L09429	Exon 6	635-636	CTATGATGAGAA-ATCAACCGGAGG	0.3 kb
202	07955-L07737	Exon 7	7 nt after exon 7, reverse	GACGTCCTGGAT-ACTCACAGGTGC	0.5 kb
	No probe	Exon 8			
148	07956-L07738	Exon 9	17 nt after exon 9	TATCCCCAGCAA-GAAGATACCATC	0.6 kb
	No probe	Exon 10			
221	07957-L07739	Exon 11	20 nt before exon 11	GGCCCTTCCTTG-TCTTCTTCATCT	0.5 kb
427	12248-L14813	Exon 12	13 nt after exon 12, reverse	CATGATGTTCAG-ACAGCCTCTTAC	0.2 kb
	No probe	Exon 13			
312	07959-L09424	Exon 14	20 nt before exon 14	TCTCATCTGACT-TCTCTTGGTTTG	0.5 kb
	No probe	Exon 15			
238	07960-L07742	Exon 16	1179-1180	GCTGTTGGTGCT-AAGGTGAGACCC	0.4 kb
	No probe	Exon 17			
160	07961-L07743	Exon 18	1285-1286	TGCCACAGGGAA-ACCCTGGTGCTG	0.4 kb
	No probe	Exon 19			
328	07962-L07744	Exon 20	1464-1465	GGAGACACTGGT-GCTAAGGGAGAG	0.3 kb
263	07963-L07745	Exon 21	1536-1537	GGAAAGCGAGGA-GCTCGAGGTGAA	0.3 kb
	No probe	Exon 22			
398	07964-L07746	Exon 23	16 nt before exon 23	AACTGTTCCTGT-GACTTCCCCCAA	0.5 kb
	No probe	Exon 24			
172	07965-L07747	Exon 25	1870-1871	GTGTGATGGGAT-TCCCTGGACCTA	1.1 kb
	No probe	Exon 26			
355	07966-L09425	Exon 27	1953-1954	CTGCAGGGTCCT-GCTGGCAAAGAT	0.4 kb
	No probe	Exon 28			
283	07967-L07749	Exon 29	2096-2097	TGAAGCAGGCAA-ACCTGGTGAACA	0.5 kb
142	07968-L07957	Exon 30	3 nt after exon 30	GGAGCAAGAGTA-AGTAGGCCTCTC	0.5 kb
	No probe	Exon 31			
411	21675-L32137	Exon 32	3 nt before exon 32	TTGGTTGTCACA-TAGGGTGATGCT	1.0 kb
	No probe	Exon 33			
419	07971-L14814	Exon 34	30 nt after exon 34	TTAAGACCCCAT-ACTTGGCCCTTC	0.4 kb
	No probe	Exon 35			
154	07972-L07753	Exon 36	2601-2600, reverse	GGTTCGCCTTTA-GCACCAGGTTGG	0.4 kb
	No probe	Exon 37			
215	07973-L09423	Exon 38	13 nt before exon 38	CTGTTCCTATGT-TCTCTCCTTCCA	0.5 kb
	No probe	Exon 39			
229	07974-L07755	Exon 40	2987-2986, reverse	GCTGTCCAGCAA-TACCTTGAGGCC	0.5 kb
	No probe	Exon 41			
436	07975-L07756	Exon 42	20 nt after exon 42	GCAACACTCCAT-GACCACAGCCTT	0.2 kb
166	07976-L07757	Exon 43	20 nt after exon 43	CTGGGCTCCAGT-TCCCTGTACCTG	0.6 kb
	No probe	Exon 44			
306	07977-L14627	Exon 45	3440-3441	ACAGGGCGACAG-AGGCATAAAGGG	0.4 kb
247	07978-L07759	Exon 46	3512-3511, reverse	CAGAGGGACCTT-GTTCACCAGGAG	0.5 kb
322	07979-L14628	Exon 47	1 nt after exon 47	GCTGGTCCTGTT-GTATGTAGCCCC	0.2 kb
274	12246-L13183	Exon 48	3791-3790, reverse	CACGAACCACAT-TGGCATCATCAG	0.4 kb
178	07981-L29327	Exon 49	4088-4089	CCCCAAGGACAA-GAGGCATGTCTG	0.4 kb
346	07982-L07763	Exon 50	4181-4180, reverse	GCAGGAAGGTCA-GCTGGATGGCCA	0.9 kb
363	07983-L07764	Exon 51	4955-4956	AAGACACAGGAA-ACAATGTATTGT	
		stop codon	*4519-4521 (Exon 51)*		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

± SNP rs72667018 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Related SALSA MLPA probemixes

P272 COL1A2 Contains probes for the *COL1A2* gene, also involved in osteogenesis imperfecta.
P452 PLS3 Contains probes for the *PLS3* gene, involved in X-linked osteoporosis.

References

- 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 P271 COL1A1

- Andersson K et al. (2017). Mutations in COL1A1 and COL1A2 and dental aberrations in children and adolescents with osteogenesis imperfecta–A retrospective cohort study. *PLoS One*, 12(5).
- Kanno J et al. (2018). Responsiveness to pamidronate treatment is not related to the genotype of type I collagen in patients with osteogenesis imperfecta. *J Bone Miner Metab*, 36(3), 344-351.
- Li L et al. (2019). Genotypic and phenotypic characterization of Chinese patients with osteogenesis imperfecta. *Hum mutat*, 40(5), 588-600.
- Lindahl K et al. (2016). Decreased fracture rate, pharmacogenetics and BMD response in 79 Swedish children with osteogenesis imperfecta types I, III and IV treated with Pamidronate. *Bone*, 87, 11-18.
- Malmgren B et al. (2017). Tooth agenesis in osteogenesis imperfecta related to mutations in the collagen type I genes. *Oral dis*, 23(1), 42-49.
- Mauri L et al. (2016). Expanding the clinical spectrum of COL1A1 mutations in different forms of glaucoma. *Orphanet J Rare Dis*, 11(1), 108.
- Takagi M et al. (2015). Severe osteogenesis imperfecta caused by double glycine substitutions near the amino-terminal triple helical region in COL1A2. *Am J Med Genet Part A*, 167(7), 1627-1631.

P271 Product history	
Version	*Modification*
B5	One reference probe has been replaced, one reference probe has been removed, and one probe length has been adjusted.
B4	Three reference probes have been replaced and one probe has been adjusted in length.
B3	Length of two probes has been adjusted.
B2	Three reference probes have been replaced, QDX2 fragments have been added.
B1	Four extra control fragments have been included at 88-96-100-105 nt. Two reference probes have been added and four probes have been replaced.
A1	First release.

Implemented changes in the product description
Version B5-01 — 17 March 2020 (02P) - 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).

- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 10 (55) – 09 June 2016
- 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 09 (55) – 06 April 2016
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 08 (55) – 05 April 2016
- Added information on page 1 about this Product Description version.
- Length of two probes adjusted in Table 1 & 2.
- References updated.

Version 07 (48)
- Electropherogram picture of old buffer (introduced Dec. 2012) removed.

Version 06 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 05 (48)
- 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.

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