Product Description

SALSA® MLPA® Probemix P381-A4 COL11A1 mix 1 & P382-B1 COL11A1 mix 2

To be used with the MLPA General Protocol.

P381 version A4.
As compared to version A3, eight reference probes have been replaced and one reference probe has been removed. For complete product history see page 9.

P382 version B1.
As compared to version A3, one COL11A1 probe has been removed, five reference probes have been replaced and two probe lengths have been adjusted. For complete product history see page 9.

Catalogue numbers:

- **P381-025R:** SALSA MLPA Probemix P381 COL11A1 mix 1, 25 reactions.
- **P381-050R:** SALSA MLPA Probemix P381 COL11A1 mix 1, 50 reactions.
- **P381-100R:** SALSA MLPA Probemix P381 COL11A1 mix 1, 100 reactions.

- **P382-025R:** SALSA MLPA Probemix P382 COL11A1 mix 2, 25 reactions.
- **P382-050R:** SALSA MLPA Probemix P382 COL11A1 mix 2, 50 reactions.
- **P382-100R:** SALSA MLPA Probemix P382 COL11A1 mix 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.

General information
The SALSA MLPA Probemixes P381 COL11A1 mix 1 and P382 COL11A1 mix 2 are **research use only (RUO)** assays for the detection of deletions or duplications in the *COL11A1* gene, which is associated with Marshall and type II Stickler syndromes.

Marshall syndrome is an autosomal dominant inherited disease characterised by short stature, nearsightedness, hearing loss, and intracranial ossifications. Type II Stickler syndrome patients have similar symptoms but are of near normal height and exhibit no bony overgrowths. Both syndromes occasionally present with cleft palate and patients frequently develop early osteoarthritis (Kahler et al. 2008). Since the characteristics of these syndromes overlap, it has been argued whether they are distinct entities or different manifestations of a single syndrome. Defects in *COL11A1* gene cause Marshall and type II Stickler syndromes.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1302/.

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 *COL11A1* exon numbering used in this P381 COL11A1 mix 1 and P382 COL11A1 mix 2 product description is the exon numbering from the NG_008033.2 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 NG 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 P381-A4 COL11A1 mix 1 contains 39 MLPA probes with amplification products between 130 and 454 nucleotides (nt) and SALSA MLPA Probemix P382-B1 COL11A1 mix 2 contains 37 MLPA probes with amplification products between 136 and 463 nt. These include a total of 56 probes that target 55 out of the 67 exons of the *COL11A1* gene. In addition, ten reference probes are included in P381-A4 and ten reference probes are included in P382-B1 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).

These probemixes contain 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 Marshall or type II Stickler syndromes. 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 *COL11A1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P381/P382 COL11A1.
- 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.

LOVD mutation database

https://databases.lovd.nl/shared/genes/COL11A1. 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 *COL11A1* exons 7 and 9 but not exon 8) to MRC Holland: info@mrcholland.com.

Table 1a. SALSA MLPA Probemix P381-A4 COL11A1 mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*COL11A1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 13351-L14781	8q	
136	**COL11A1 probe** 13224-L14557		**Exon 28**
142	**COL11A1 probe** 13225-L14558		**Exon 33**
148	**COL11A1 probe** 13226-L14559		**Exon 62**
154 *	Reference probe 05751-L05189	5p	
160	**COL11A1 probe** 13227-L14560		**Exon 26**
172	**COL11A1 probe** 13229-L14562		**Exon 3**
178 *	Reference probe 15867-L17960	2p	
184	**COL11A1 probe** 13228-L26097		**Exon 40**
190	**COL11A1 probe** 13231-L14564		**Exon 21**
196	**COL11A1 probe** 13230-L26098		**Exon 41**
204	**COL11A1 probe** 13232-L26099		**Exon 53**
211	**COL11A1 probe** 13233-L26100		**Exon 66**
220	**COL11A1 probe** 13234-L14567		**Exon 1**
232	**COL11A1 probe** 13235-L14568		**Exon 24**
238	**COL11A1 probe** 21480-L30139		**Exon 8**
244	**COL11A1 probe** 13237-L14570		**Exon 49**
256	**COL11A1 probe** 13238-L14571		**Exon 18**
268 *	Reference probe 17398-L19176	3p	
274	**COL11A1 probe** 13240-L14573		**Exon 11**
283 *	Reference probe 22489-L31638	14q	
292	**COL11A1 probe** 13241-L14574		**Exon 7**
301	**COL11A1 probe** 13242-L14575		**Exon 44**
309	**COL11A1 probe** 13243-L14576		**Exon 56**
319 *	Reference probe 13345-L14771	18q	
328	**COL11A1 probe** 13244-L14577		**Exon 35**
337	**COL11A1 probe** 13245-L14578		**Exon 58**
346	**COL11A1 probe** 13246-L14579		**Exon 20**
355 *	Reference probe 16563-L19054	11q	
364	**COL11A1 probe** 13247-L14580		**Exon 16**
373	**COL11A1 probe** 13248-L14581		**Exon 30**
382 *	Reference probe 12940-L14808	13q	
391 *	Reference probe 19098-L24985	4q	
400	**COL11A1 probe** 13250-L14583		**Exon 9**
407	**COL11A1 probe** 13251-L14584		**Exon 29**
417	**COL11A1 probe** 13252-L14585		**Exon 51**
433	**COL11A1 probe** 13253-L14586		**Exon 57**
445	**COL11A1 probe** 13254-L14587		**Exon 14**
454	Reference probe 10685-L11267	6p	

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

* New in version A4.

Table 1b. SALSA MLPA Probemix P382-B1 COL11A1 mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*COL11A1*
64-105	Control fragments – see table in probemix content section for more information		
136 *	Reference probe 21700-L30358	15q	
142	**COL11A1 probe** 13256-L14589		**Exon 4**
148	**COL11A1 probe** 13257-L14590		**Exon 59**
154 *	Reference probe 21201-L29576	9p	
160	**COL11A1 probe** 13258-L14591		**Exon 34**
166	**COL11A1 probe** 13259-L14592		**Exon 64**
175	Reference probe 00808-L00638	18q	
184	**COL11A1 probe** 13261-L14594		**Exon 43**
190	**COL11A1 probe** 13262-L14595		**Exon 25**
197	**COL11A1 probe** 13263-L14596		**Exon 60**
202 *	Reference probe 12566-L13616	11p	
208	**COL11A1 probe** 13264-L14597		**Exon 17**
214	**COL11A1 probe** 13265-L15166		**Exon 38**
220	**COL11A1 probe** 13266-L14599		**Exon 54**
232	**COL11A1 probe** 13267-L14600		**Intron 5**
238	**COL11A1 probe** 13268-L14601		**Exon 61**
244	**COL11A1 probe** 13269-L14602		**Exon 19**
256	**COL11A1 probe** 13270-L14603		**Exon 67**
265	**COL11A1 probe** 13271-L14604		**Exon 39**
283 *	Reference probe 21887-L31064	21q	
292	**COL11A1 probe** 13273-L14606		**Exon 27**
301	**COL11A1 probe** 13274-L14607		**Exon 10**
310	**COL11A1 probe** 13275-L14608		**Exon 37**
328	**COL11A1 probe** 13276-L14609		**Exon 15**
337	Reference probe 07132-L06741	2p	
346	**COL11A1 probe** 13278-L14611		**Exon 42**
355 *	Reference probe 11614-L12374	12p	
363	**COL11A1 probe** 13279-L14612		**Exon 2**
373	**COL11A1 probe** 13280-L14613		**Exon 46**
382	Reference probe 06549-L06107	5q	
399	**COL11A1 probe** 13282-L14615		**Exon 6**
409	**COL11A1 probe** 13283-L14616		**Exon 22**
418	Reference probe 06876-L05967	3p	
436	**COL11A1 probe** 13285-L14618		**Exon 5**
449 ¥	**COL11A1 probe** 13286-L32473		**Exon 48**
456 ¥	**COL11A1 probe** 13287-L32472		**Exon 63**
463	Reference probe 10108-L10532	8q	

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

* New in version B1.
¥ Changed in version B1. Minor alteration, no change in sequence detected.

Table 2. COL11A1 probes arranged according to chromosomal location

Length (nt) P381	Length (nt) P382	SALSA MLPA probe	*COL11A1* exon[a]	Ligation site NM_080629.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*319-321 (Exon 1)*		
220		13234-L14567	Exon 1	155-156	ACACGAAGAACG-CAAACCATCAAA	25.4 kb
	363	13279-L14612	Exon 2	492-493	TCAAAAACAACG-GGATTTTGCACA	4.1 kb
172		13229-L14562	Exon 3	614-615	CCCAGAAGACTT-TTCAATACTATT	4.1 kb
	142	13256-L14589	Exon 4	823-824	ATCGGGTAGCAA-TCAGCGTGGAGA	43.5 kb
	436	13285-L14618	Exon 5	994-995	AGTTTTTGATCA-CAGGTGATCCCA	4.9 kb
	232	13267-L14600	Intron 5	401 nt before exon 6	CAATTGCCTGAT-ATTTTTTCCTTT	0.5 kb
	399	13282-L14615	Exon 6	1231-1232	AAGCATCAGCAA-AAGCCAAACTAG	0.3 kb
292		13241-L14574	Exon 7	1310-1311	AAGTTACCAGAC-AGAAGCTCCTAG	2.7 kb
238		21480-L30139	Exon 8	1465-1466	GCAGGGATTCTG-ATCTTCTGGTAG	1.1 kb
400		13250-L14583	Exon 9	1618-1619	GCCATGGTGCAT-ATGGAGAGAAAG	2.9 kb
	301	13274-L14607	Exon 10	1677-1678	ATGCTTGTCGAA-GGACCACCAGGA	1.0 kb
274		13240-L14573	Exon 11	1722-1723	ATGGGTCCTCCA-GGTCTACAAGGC	5.4 kb
		No probe	Exon 12			
		No probe	Exon 13			
445		13254-L14587	Exon 14	1962-1963	CCAATGGGTCTA-ACTGGAAGACCA	3.9 kb
	328	13276-L14609	Exon 15	1995-1996	GGGGGGCCTGGT-TCATCTGGGGCC	2.3 kb
364		13247-L14580	Exon 16	2 nt after exon 16	GGAAAAAGGGTA-TGGCTTATTTTC	0.1 kb
	208	13264-L14597	Exon 17	9 nt before exon 17	TCATGCTGTTGG-CATTATTAGGGT	0.3 kb
256		13238-L14571	Exon 18	2181-2182	CCGGGTCTGCCA-GGTGACAAAGGT	1.2 kb
	244	13269-L14602	Exon 19	2249-2250	TGATGATGGAAT-GAGGGTATGTTA	0.1 kb
346		13246-L14579	Exon 20	2274-2275	GGAGAAATTGGA-CCAAGAGGTCTT	1.2 kb
190		13231-L14564	Exon 21	2328-2329	GGTCCAAGGGGA-ACTCCAGGAGCT	0.4 kb
	409	13283-L14616	Exon 22	2359-2360	TGCAGGGTATGG-CAGGTGTAGATG	0.9 kb
		No probe	Exon 23			
232		13235-L14568	Exon 24	2496-2497	CCTGGTGAAAAA-GTAAGTTACTCT	3.6 kb
	190	13262-L14595	Exon 25	2521-2522	AACCAGGACTTG-CTGGACTTCCTG	1.2 kb
160		13227-L14560	Exon 26	2582-2583	CCAGTCTGGAGA-AAAGGGGGCTCT	1.0 kb
	292	13273-L14606	Exon 27	16 nt before exon 27	CGTATAAGTGGT-AATTCATCTATT	0.2 kb
136		13224-L14557	Exon 28	2665-2666	CAGATGGTGTCA-GAGGTCTCAAGG	6.4 kb
407		13251-L14584	Exon 29	2736-2737	GACATGGGTCTA-AAAGGTGACAGA	1.8 kb
373		13248-L14581	Exon 30	2796-2797	GGCCCTGAAGGA-CCCAAAGGTCGA	8.6 kb
		No probe	Exon 31			
		No probe	Exon 32			
142		13225-L14558	Exon 33	2979-2980	GTAGCTGGCAAA-CCAGGCCCTCGG	0.2 kb
	160	13258-L14591	Exon 34	3021-3022	GGTCCTCGAGGT-TCAAGAGGTGCA	0.2 kb
328		13244-L14577	Exon 35	3072-3073	CAGGGCACTTCA-GGTGGCGATGGC	8.5 kb
		No probe	Exon 36			
	310	13275-L14608	Exon 37	3207-3208	CCTGGGCAACGT-GGGGAGACTGTA	4.7 kb
	214	13265-L15166	Exon 38	3219-3220	CCTCCTTAGGGA-TTTCAAGGCAAG	2.8 kb
	265	13271-L14604	Exon 39	3282-3283	GGACCAACCGGT-GAGACTGGTCCA	0.5 kb
184		13228-L26097	Exon 40	3395-3396	TCCAGGTCCTCA-AGGTATCTCAGG	0.4 kb
196		13230-L26098	Exon 41	3489-3490	GGACTGAAAGGA-GGGGAAGGTCCC	15.0 kb
	346	13278-L14611	Exon 42	3543-3544	GGAGAACGTGGG-TCAGCAGGTACA	6.6 kb
	184	13261-L14594	Exon 43	3684-3685	CAAGGTCCTGTT-GGTCTCCCAGGG	1.3 kb
301		13242-L14575	Exon 44	3744-3745	TTTCAGGGTGAA-ATTGGTGAGCCG	4.6 kb
		No probe	Exon 45			
	373	13280-L14613	Exon 46	3886-3887	GACAGCAGGGGA-TGTTTGGGCAAA	13.0 kb
		No probe	Exon 47			

Length (nt) P381 P382	SALSA MLPA probe	*COL11A1* exon[a]	Ligation site NM_080629.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
449	13286-L32473	Exon 48	4053-4054	CAAGGTCCCAAT-GGAGCTGATGTA	1.2 kb
244	13237-L14570	Exon 49	4113-4114	GGTGTTGGAGAA-AAGGTAAATATG	5.5 kb
	No probe	Exon 50			
417	13252-L14585	Exon 51	4204-4205	AGAAAGGGGAAG-CTGGTCCACCTG	1.1 kb
	No probe	Exon 52			
204	13232-L26099	Exon 53	4371-4372	GGTGAAGATGGA-GATCCTGGTCAA	1.5 kb
220	13266-L14599	Exon 54	4426-4427	GCCCACCAGGTC-CTCCTGGAAAAC	13.4 kb
	No probe	Exon 55			
309	13243-L14576	Exon 56	4524-4525	GGTCCTCCTGGA-AAAACCGGCCCA	0.6 kb
433	13253-L14586	Exon 57	4644-4645	CAAGATGGACCA-CCTGGTCCTATG	7.7 kb
337	13245-L14578	Exon 58	4686-4687	GGTCTCAAAGGT-GACCCTGGCTCC	0.9 kb
148	13257-L14590	Exon 59	4726-4727	ATCCTGGTTTAA-TTGGCCTGATTG	0.6 kb
197	13263-L14596	Exon 60	4822-4823	CTCTCTAGGGAA-TTCCTGGTCCTG	0.2 kb
238	13268-L14601	Exon 61	4894-4895	GCCCAAAGGGTA-ACAAAGGCTCTA	0.1 kb
148	13226-L14559	Exon 62	4915-4916	AACAGGGACCCG-CTGGCCAGAAAG	1.7 kb
456	13287-L32472	Exon 63	5044-5045	GCATGCAAGCAG-ATGCAGATGATA	3.7 kb
166	13259-L14592	Exon 64	5247-5248	CAAGGTTGCTCA-GGAGATTCCTTC	3.4 kb
	No probe	Exon 65			
211	13233-L26100	Exon 66	5462-5463	GAAACTTCTGAC-TGCCTCTGCTCG	1.7 kb
256	13270-L14603	Exon 67	5670-5671	GAAATCAATACA-CCAAAAATTGAT	
	stop codon 5773-5775 (Exon 67)				

[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.

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

P214 COL2A1 Contains probes for *COL2A1*, involved in Stickler syndrome type I.

References

- Kahler RA et al. (2008). Collagen 11a1 is indirectly activated by lymphocyte enhancer-binding factor 1 (Lef1) and negatively regulates osteoblast maturation. *Matrix biol*, 27(4), 330-338.
- 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 P381/P382 COL11A1

- Vijzelaar R et al. (2013). Deletions within COL11A1 in Type 2 Stickler syndrome detected by multiplex ligation-dependent probe amplification (MLPA). *BMC Med Genet* 14:48.

P381 product history	
Version	*Modification*
A4	Eight reference probes have been replaced and one reference probe has been removed.
A3	One reference probes has been removed and one probe length has been adjusted.
A2	Two reference probes have been replaced and the control fragments have been adjusted (QDX2).
A1	First release.

P382 product history	
Version	*Modification*
B1	One COL11A1 probe has been removed, five reference probes have been replaced and two probe lengths have been adjusted.
A3	Four reference probes have been replaced and the control fragments have been adjusted (QDX2).
A2	One reference probe has been removed.
A1	First release.

Implemented changes in the product description

Version A4/B1-01– 30 April 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).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 08 – 20 June 2017 (55)
- 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).
- Small changes of probe lengths in Tables 1 and 2 in order to better reflect the true lengths of the amplification products.
- Exon numbering of the *COL11A1* gene has been changed in Tables 1 and 2.
- Various minor textual changes on page 1 and 2.

Version 07 (53)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 06 (49)
- Reference added about P381 and P382 probemixes.

Version 05 (48)
- Warning added in Table 1, 130 nt probe 09017-L09271; 154 nt probe 07038-L06649; 382 nt probe 09971-L10730; 427 nt probe 10297-L10809.

Version 04 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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