Product Description SALSA® MLPA® Probemix P331-B2 COL5A1 MIX-1 & P332-C2 COL5A1 MIX-2

To be used with the MLPA General Protocol.

P331 Version B2. As compared to version B1, five reference probes have been replaced. For complete product history see page 9.
P332 Version C2. As compared to version C1, 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 9.

Catalogue numbers:
- **P331-025R:** SALSA MLPA Probemix P331 COL5A1 MIX-1, 25 reactions.
- **P331-050R:** SALSA MLPA Probemix P331 COL5A1 MIX-1, 50 reactions.
- **P331-100R:** SALSA MLPA Probemix P331 COL5A1 MIX-1, 100 reactions.

- **P332-025R:** SALSA MLPA Probemix P332 COL5A1 MIX-2, 25 reactions.
- **P332-050R:** SALSA MLPA Probemix P332 COL5A1 MIX-2, 50 reactions.
- **P332-100R:** SALSA MLPA Probemix P332 COL5A1 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.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 Probemixes P331 COL5A1 MIX-1 and P332 COL5A1 MIX-2 are a **research use only (RUO)** assays for the detection of deletions or duplications in the *COL5A1* gene, which is associated with classic Ehlers-Danlos syndromes.

Ehlers-Danlos syndromes (EDS) are a group of heritable connective tissue disorders characterised by hyperextensible skin, articular/joint hypermobility and soft tissue fragility. The classic type of EDS, formly known as type I and II, is caused by mutations in the type V collagen genes *COL5A1* and *COL5A2* and is inherited in an autosomal dominant manner. Mutations of *COL5A1* account for 75-78% of classic EDS. Studies suggest that type V collagen controls the assembly of heterotypic fibres composed of type I and type V collagen as well as the assembly of other collagen types.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1244/

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 *COL5A1* exon numbering used in this P331-B2/P332-C2 COL5A1 product description is the exon numbering from the RefSeq transcript NM_000093.5, which is identical to the LRG_737 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 P331-B2 COL5A1 MIX-1 contains 40 MLPA probes with amplification products between 130 and 463 nucleotides (nt, Table 1a). The SALSA MLPA Probemix P332-C2 COL5A1 MIX-2 contains 36 MLPA probes with amplification products between 130 and 454 nt (Table 1b).

P331-B2 and P332-C2 contain 29 and 27 probes for the *COL5A1* gene, respectively. Together, these probemixes cover 56 out of 66 *COL5A1* exons by at least one probe (Table 2). In addition, eleven and nine reference probes are included in P331-B2 and P332-C2, respectively, 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).

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.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 classic Ehlers-Danlos syndromes. 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 *COL5A1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemixes P331/P332 COL5A1.
- 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.

***COL5A1* mutation database:** https://databases.lovd.nl/shared/genes/COL5A1. 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 *COL5A1* exons 13 and 15 but not exon 14) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P331-B2 COL5A1 MIX-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	COL5A1
64-105	Control fragments – see table in probemix content section for more information		
130 *	Reference probe 21397-L29874	3q22	
135 «	**COL5A1 probe** 10374-L10926		**Exon 1**
142	**COL5A1 probe** 10400-L10952		**Exon 56**
148	**COL5A1 probe** 10381-L12251		**Exon 15**
154	Reference probe 13816-L15310	2q13	
160	**COL5A1 probe** 15046-L16803		**Exon 31**
166	**COL5A1 probe** 10398-L20610		**Exon 47**
172	**COL5A1 probe** 13020-L14186		**Exon 22**
178	Reference probe 10676-L20612	6p12	
190	**COL5A1 probe** 17088-L20200		**Exon 65**
195	**COL5A1 probe** 10375-L16562		**Exon 2**
201	Reference probe 12681-L13759	7q11	
208	**COL5A1 probe** 10377-L10929		**Exon 7**
214	**COL5A1 probe** 10380-L10932		**Exon 14**
220	**COL5A1 probe** 12070-L13714		**Exon 44**
232	**COL5A1 probe** 12643-L10935		**Exon 18**
238 Ж	**COL5A1 probe** 15047-SP0243-L16804		**Exon 37**
244	**COL5A1 probe** 10384-L10936		**Exon 19**
250	Reference probe 13188-L06519	11q12	
258	**COL5A1 probe** 10401-L16563		**Exon 58**
265	**COL5A1 probe** 10385-L10937		**Exon 21**
274	**COL5A1 probe** 10403-L10955		**Exon 60**
285 *	Reference probe 08666-L22903	9q31	
301	**COL5A1 probe** 16866-L19659		**Exon 64**
310	**COL5A1 probe** 16868-L19661		**Exon 16**
319	Reference probe 11333-L12058	12p13	
337	**COL5A1 probe** 10376-L10928		**Exon 4**
344	**COL5A1 probe** 16871-L19664		**Exon 24**
355 *	Reference probe 14661-L16313	20q13	
364	**COL5A1 probe** 10397-L10949		**Exon 45**
373	**COL5A1 probe** 10393-L12863		**Exon 35**
382	**COL5A1 probe** 10399-L10951		**Exon 50**
393 *	Reference probe 19019-L24832	21q22	
400	**COL5A1 probe** 12638-L10941		**Exon 27**
409	**COL5A1 probe** 19769-L28313		**Exon 13**
420	**COL5A1 probe** 12193-L13120		**Exon 10**
427 *	Reference probe 15893-L24326	2p16	
436	**COL5A1 probe** 10395-L10947		**Exon 41**
445	**COL5A1 probe** 10391-L10943		**Exon 32**
463	Reference probe 09908-L10321	16p13	

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

* New in version B2.
« 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.
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.

Table 1b. SALSA MLPA Probemix P332-C2 COL5A1 MIX-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	COL5A1
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L19287	5q31	
142	**COL5A1 probe** 10415-L19072		**Exon 20**
151	**COL5A1 probe** 12658-L20541		**Exon 42**
157	Reference probe 13816-L20542	2q13	
165	**COL5A1 probe** 15327-L20543		**Exon 28**
175	**COL5A1 probe** 10416-L12862		**Exon 23**
188	**COL5A1 probe** 10437-L10989		**Exon 61**
195	**COL5A1 probe** 10432-L19073		**Exon 52**
200	**COL5A1 probe** 10409-L19074		**Exon 6**
208	Reference probe 10730-L11312	6p12	
215	**COL5A1 probe** 10424-L12859		**Exon 38**
220	**COL5A1 probe** 10433-L12860		**Exon 53**
232	**COL5A1 probe** 10410-L12202		**Exon 8**
238	**COL5A1 probe** 10438-L10990		**Exon 62**
257	**COL5A1 probe** 10436-L19067		**Exon 57**
265	**COL5A1 probe** 10417-L10969		**Exon 25**
274	**COL5A1 probe** 10419-L10971		**Exon 29**
292	**COL5A1 probe** 10435-L10987		**Exon 55**
300	Reference probe 16027-L18204	12p13	
308	**COL5A1 probe** 10414-L19075		**Exon 17**
315	**COL5A1 probe** 10439-L19076		**Exon 63**
325 *	Reference probe 11002-L24656	4q22	
334	**COL5A1 probe** 10428-L19077		**Exon 46**
341 ¥	**COL5A1 probe** 21528-L32145		**Exon 5**
348 Ж	**COL5A1 probe** 15620-SP0245-L19079		**Exon 51**
358	**COL5A1 probe** 16808-L19980		**Exon 3**
373	**COL5A1 probe** 10421-L10973		**Exon 34**
383	Reference probe 00973-L13269	10q21	
391	Reference probe 18069-L22459	16q23	
400	**COL5A1 probe** 10411-L12204		**Exon 9**
409	**COL5A1 probe** 10427-L10979		**Exon 43**
418	**COL5A1 probe** 10394-L10946		**Exon 40**
427	Reference probe 11532-L16599	18q22	
436	**COL5A1 probe** 15619-L17480		**Exon 11**
445	**COL5A1 probe** 14803-L16510		**Exon 30**
454	Reference probe 15515-L17370	7q32	

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

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.
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.

Table 2. *COL5A1* probes arranged according to chromosomal location

Length (nt) P331	Length (nt) P332	SALSA MLPA probe	COL5A1 exon[a]	Ligation site NM_000093.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*386-388 (Exon 1)*		
135«		10374-L10926	Exon 1	239-240	CGCCGCCACAAA-GAAGAACGGGGG	49.0 kb
195		10375-L16562	Exon 2	597-598	TTCCAAAGGCCC-GGATGTCGCTTA	8.9 kb
	358	16808-L19980	Exon 3	700-701	ATCCTAACAACT-GTGAAAGCCAAG	1.3 kb
337		10376-L10928	Exon 4	926-927	TCACCTTGATCC-TCGACTGTAAAA	26.1 kb
	341	21528-L32145	Exon 5	1074-1075	TGTCTCGGACCA-CCGGGCAGCTTA	1.4 kb
	200	10409-L19074	Exon 6	1203-1204	GGGTGAGACCTA-TTACTACGAATA	1.6 kb
208		10377-L10929	Exon 7	1422-1423	CAGTGAGGACTA-CTACACGCCCTC	1.2 kb
	232	10410-L12202	Exon 8	1631-1632	TTGACGAGAACT-ACTACGACCCCT	0.5 kb
	400	10411-L12204	Exon 9	1741-1742	CGGGGCGAGAAA-GGCCAAAAGGGA	6.5 kb
420		12193-L13120	Exon 10	24 nt after exon 10	TTTATCCTGTGA-CTTGCAGAAGGT	0.3 kb
	436	15619-L17480	Exon 11	25 nt after exon 11	TGTGTTTCCTGA-GATCACACAAGG	11.9 kb
		no probe	Exon 12			
409		19769-L28313	Exon 13	26 nt before exon 13	TCACTGCTCCCA-GAGTGACCCTTG	1.9 kb
214		10380-L10932	Exon 14	2086-2087	ATGGGTCTCACA-GGGAGACCTGGC	1.2 kb
148		10381-L12251	Exon 15	2122-2121; reverse	CCCTTCAAACCT-CCGCTCCCAGGG	0.4 kb
310		16868-L19661	Exon 16	35 nt before exon 16	GAGAAAGGCGGA-CTCGCCACTGAC	2.6 kb
	308	10414-L19075	Exon 17	2252-2253	GAATGCCTGGAC-AAACTGGCCCCA	1.5 kb
232		12643-L10935	Exon 18	2290-2291	TTCGACGGCCTG-GCTGGGTTGCCA	3.7 kb
244		10384-L10936	Exon 19	2371-2372	GACGATGGAGAA-AGGGTAGGTATT	1.7 kb
	142	10415-L19072	Exon 20	2377-2378	CCTCTGCAGGGT-GACGACGGAGAA	2.0 kb
265		10385-L10937	Exon 21	20 nt before exon 21	GTTTAAATCCTA-TTTTCCCTTTCC	0.8 kb
172		13020-L14186	Exon 22	2486-2487	GTGTCACGGGTA-TGGACGGCCAGC	0.5 kb
	175	10416-L12862	Exon 23	18 nt before exon 23	CTTCAGTGCCTT-TGCTCTTGTCTC	0.5 kb
344		16871-L19664	Exon 24	87 nt after exon 24	CTGGTATCCACC-AGCTCTCAATGT	1.0 kb
	265	10417-L10969	Exon 25	2657-2658	GAATGCCCGGTG-CTGACGGACCCC	6.5 kb
		no probe	Exon 26			
400		12638-L10941	Exon 27	2761-2762	CCAGGTCCTCGA-GGAGTCAAGGTG	5.2 kb
	165	15327-L20543	Exon 28	2804-2805	TGAAGGGCACAA-AGGGCGAGAAGG	2.6 kb
	274	10419-L10971	Exon 29	2845-2846	GGGTTTAAAGGA-GACATGGGCATC	2.4 kb
	445	14803-L16510	Exon 30	2935-2934; reverse	TCACCATTGGGA-CCTCCGCGACCC	1.0 kb
160		15046-L16803	Exon 31	3028-3029	AGACAAGGACCA-AAGGTAACTTCT	3.1 kb
445		10391-L10943	Exon 32	3034-3035	CTCTTTCAGGGC-TCTATTGGATTC	6.1 kb
		no probe	Exon 33			
	373	10421-L10973	Exon 34	5 nt before exon 34	CTCTCCCATCTG-TCCAGGGTCCGA	1.1 kb
373		10393-L12863	Exon 35	3209-3210	GTGACGGCCCAG-CTGGCCCTCCTG	2.0 kb
		no probe	Exon 36			
238 Ж		15047-SP0243-L16804	Exon 37	1 nt before exon 37; 3312-3313	GTTTTTTCTTCA-30 nt spanning oligo-ACACCCTGGACA	3.6 kb
	215	10424-L12859	Exon 38	3 nt after exon 38	GGCCCTCAGGTA-AGCTCCAGCCTT	3.0 kb
		no probe	Exon 39			
	418	10394-L10946	Exon 40	5 nt before exon 40	TCTGACTCTGTT-TTCAGGGTGACC	0.2 kb
436		10395-L10947	Exon 41	3595-3596	TTGCAGGGAGCT-CTTGGACTGAAA	1.1 kb
	151	12658-L20541	Exon 42	17 nt after exon 42	TACTGCCTTGGA-TTGGGGGAGCCC	3.0 kb
	409	10427-L10979	Exon 43	9 nt after exon 43	AAGGTAAGGCAA-ATCCAGAGTGAC	1.0 kb
220		12070-L13714	Exon 44	8 nt after exon 44	ACAGGTAAGTAT-TGGCACGGGGGC	1.1 kb
364		10397-L10949	Exon 45	3952-3953	GGCCCCATCGGA-CAGCCAGGCCCC	0.2 kb
	334	10428-L19077	Exon 46	4034-4035	GTGATGAAGGTC-CCAGAGGCTTTC	0.9 kb
166		10398-L20610	Exon 47	4093-4094	CCAGGACCTCCA-GGCGAGAAGGGT	2.3 kb
		no probe	Exon 48			
		no probe	Exon 49			
382		10399-L10951	Exon 50	9 nt before exon 50	ACACTGGCCTCT-TTCCTCCAGGGA	0.7 kb
	348Ж	15620-SP0245-L19079	Exon 51	4405-4404; reverse; 24 nt after exon 51	GGAAAACCCACT-30 nt spanning oligo-CCAAGGCCATCA	0.4 kb
	195	10432-L19073	Exon 52	4479-4480	CCCTGGTGACAA-AGGAGATGATGG	1.0 kb
	220	10433-L12860	Exon 53	14 nt before exon 53	CACTCTGTTCTT-TCTCCCAATACC	1.7 kb

Length (nt) P331	Length (nt) P332	SALSA MLPA probe	COL5A1 exon[a]	Ligation site NM_000093.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		no probe	Exon 54			
	292	10435-L10987	Exon 55	4628-4627; reverse	AGGGCCTTCCAA-GCCGGCTTCTCC	0.2 kb
142		10400-L10952	Exon 56	4726-4727	GTGTTTCAGGGA-GAACAAGGTCTC	0.2 kb
	257	10436-L19067	Exon 57	4822-4823	TCTGGTCCCAAA-GGTGAAAAGGTA	1.1 kb
258		10401-L16563	Exon 58	4894-4895	AAGGGCGACCGT-GGTCTCCCTGGC	2.8 kb
		no probe	Exon 59			
274		10403-L10955	Exon 60	5016-5017	TCCAAAAGGTGC-TAAGGGCTCCTC	0.4 kb
	188	10437-L10989	Exon 61	14 nt before exon 61	GGGTTGATTCTT-TTCTTTCTCCCC	1.4 kb
	238	10438-L10990	Exon 62	5244-5245	CTCTCTGAAGCT-GGAGATTGAGCA	1.0 kb
	315	10439-L19076	Exon 63	6 nt before exon 63	CCCGTCTCTGAA-ATCCAGGTGAAT	4.3 kb
301		16866-L19659	Exon 64	16 nt after exon 64	GGTGGCCTCTGG-CGTCTTTGCGGT	5.1 kb
190		17088-L20200	Exon 65	5732-5733	ACCCCTACATCC-GCGCCCTGGTGG	
		no probe	Exon 66			
			stop codon	*5900-5902 (Exon 66)*		

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.

« 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.
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.

Related SALSA MLPA probemixes

P191/P192 Alport	Contains probes for *COL4A5*, involved in Alport syndrome and Hereditary Nephritis.
P214 COL2A1	Contains probes for *COL2A1*, involved in a number of different heritable skeletal disorders.
P271 COL1A1	Contain probes for *COL1A1*, involved in Osteogenesis imperfecta.
P272 COL1A2	Contain probes for *COL1A2*, involved in Osteogenesis imperfecta.
P381/P382 COL11A1	Primary screening for Marshall syndrome and type II Stickler syndrome.

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 Probemixes P331/P332 COL5A1

- Angwin C et al. (2019). Absence of collagen flowers on electron microscopy and identification of (likely) pathogenic COL5A1 variants in two patients. *Genes*. 10:762.
- Ritelli M et al. (2013). Clinical and molecular characterization of 40 patients with classic Ehlers–Danlos syndrome: identification of 18 COL5A1 and 2 COL5A2 novel mutations. *Orphanet J Rare Dis*. 5:58.
- Ritelli M et al. (2019). Expanding the clinical and mutational spectrum of recessive AEBP1-related classical-like Ehlers-Danlos syndrome. *Genes.* 10:135.
- Weiss et al. (2013). Best practice guidelines for the use of next-generation sequencing applications in genome diagnostics: a national collaborative study of Dutch genome diagnostic laboratories. *Hum Mutat*. 34:1313-21.

P331 Product history	
Version	*Modification*
B2	Five reference probes have been replaced.
B1	Two target probes have been removed.
A1	First release.

P332 Product history	
Version	*Modification*
C2	One reference probe has been replaced, one reference probe has been removed and one probe length has been adjusted.
C1	One target probe has been removed.
B1	One target probe has been removed, one reference probe has been added and one reference probe has been replaced.
A1	First release.

Implemented changes in the product description

Version B2/C2-01— 22 April 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).
- Ligation sites of the probes targeting the *COL5A1* gene updated according to new version of the NM_ reference sequence.

Version 04 – 06 January 2017 (55)

- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included) for P331.
- 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) for P332.

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