Product Description
SALSA® MLPA® Probemixes
P391-A3 LAMA2 mix 1 & P392-A3 LAMA2 mix 2

To be used with the MLPA General Protocol.

P391 version A3
For complete product history see page 9.

P392 version A3
For complete product history see page 9.

Catalogue numbers:
- **P391-025R:** SALSA MLPA Probemix P391 LAMA2 mix 1, 25 reactions.
- **P391-050R:** SALSA MLPA Probemix P391 LAMA2 mix 1, 50 reactions.
- **P391-100R:** SALSA MLPA Probemix P391 LAMA2 mix 1, 100 reactions.

- **P392-025R:** SALSA MLPA Probemix P392 LAMA2 mix 2, 25 reactions.
- **P392-050R:** SALSA MLPA Probemix P392 LAMA2 mix 2, 50 reactions.
- **P392-100R:** SALSA MLPA Probemix P392 LAMA2 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 P391 LAMA2 mix 1 and P392 LAMA2 mix 2 are **research use only (RUO)** assays for the detection of deletions or duplications in the *LAMA2* gene, which is associated with *LAMA2* muscular dystrophy.

LAMA2 muscular dystrophy shows a heterogeneous disease spectrum ranging from a severe congenital muscular dystrophy type 1A (MDC1A; merosin-deficient congenital muscular dystrophy type 1A) to a milder late-onset muscular dystrophy. The disease is caused by mutations in the *LAMA2* gene that encodes the laminin α2 subunit. Laminins are a family of heterotrimeric glycoproteins. The laminin α2 subunit is part of laminin-211 (also known as merosin or laminin-2) and laminin-221 (laminin-4), both of which play an important role in skeletal muscle. The *LAMA2* gene (65 exons) spans ~633 kb of genomic DNA and is located on 6q22.33, ~129 Mb from the p-telomere. *LAMA2* exons 44 and 53 are unusually small, with 6 and 12 nucleotides (nt), respectively.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK97333/.

These SALSA MLPA probemixes are not CE/FDA registered for use in diagnostic procedures. Purchase of these products 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 *LAMA2* exon numbering used in this P391-A3/P392-A3 LAMA2 product description is the exon numbering from the LRG_409 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P391-A3 LAMA2 mix 1 contains 43 MLPA probes with amplification products between 130 and 492 nt. The SALSA MLPA Probemix P392-A3 LAMA2 mix 2 contains 43 MLPA probes with amplification products between 136 and 483 nt.

The P391-A3 and P392-A3 probemixes contain 33 probes and 35 probes for the *LAMA2* gene, respectively. Together, these probemixes contain at least one probe for each exon of *LAMA2* with the exception of exons 18, 44 and 48. Two probes are present for exons 1, 2, 4 and 65, and three probes are present for exon 56. The P391-A3 and P392-A3 probemixes contain ten and eight reference probes, respectively. These reference probes detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

These probemixes 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 muscular dystrophy. 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 *LAMA2* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemixes P391 LAMA2 mix 1 and P392 LAMA2 mix 2.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***LAMA2* mutation database**

https://databases.lovd.nl/shared/genes/LAMA2. 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 *LAMA2* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P391-A3 LAMA2 mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*LAMA2*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q	
136	Reference probe 10995-L11666	4q	
142	**LAMA2 probe** 14922-L16655		**Exon 10**
148	**LAMA2 probe** 14958-L16694		**Exon 56**
160	**LAMA2 probe** 14925-L18029		**Exon 60**
172	**LAMA2 probe** 14926-L16659		**Exon 14**
178	**LAMA2 probe** 14962-L16698		**Exon 54**
184	**LAMA2 probe** 14928-L16661		**Exon 46**
192	**LAMA2 probe** 14964-L16700		**Exon 2**
196	Reference probe 10076-L10500	8q	
202	**LAMA2 probe** 14930-L16663		**Exon 3**
208	**LAMA2 probe** 14966-L16702		**Exon 6**
214	**LAMA2 probe** 14967-L28797		**Exon 24**
220	**LAMA2 probe** 14968-L16704		**Exon 49**
231	**LAMA2 probe** 14934-L16667		**Exon 45**
238	**LAMA2 probe** 14970-L16706		**Exon 30**
247	**LAMA2 probe** 14971-L16707		**Exon 55**
256	**LAMA2 probe** 14972-L16708		**Exon 1**
264	Reference probe 06007-L21163	2q	
274	**LAMA2 probe** 14938-L16671		**Exon 41**
283	**LAMA2 probe** 14939-L16672		**Exon 38**
292	**LAMA2 probe** 14940-L16673		**Exon 43**
301	**LAMA2 probe** 14976-L16712		**Exon 65**
310 Ж	**LAMA2 probe** 20119-SP0237-L16713		**Exon 5**
319	Reference probe 12552-L13602	3q	
328 Ж	**LAMA2 probe** 14978-SP0238-L16714		**Exon 28**
337 Ж	**LAMA2 probe** 14979-SP0239-L16715		**Exon 52**
346	**LAMA2 probe** 14980-L16716		**Exon 16**
355	Reference probe 16520-L28798	11p	
363	**LAMA2 probe** 14981-L16717		**Exon 4**
373	**LAMA2 probe** 14982-L16718		**Exon 37**
384	**LAMA2 probe** 14983-L16719		**Exon 20**
391	**LAMA2 probe** 14984-L16720		**Exon 62**
400	Reference probe 09998-L10457	7q	
409	**LAMA2 probe** 14950-L16683		**Exon 31**
416	**LAMA2 probe** 14986-L16722		**Exon 34**
427	**LAMA2 probe** 14987-L16723		**Exon 12**
436	**LAMA2 probe** 14988-L18028		**Exon 57**
442	Reference probe 19635-L26294	10p	
454	**LAMA2 probe** 14954-L16687		**Exon 59**
462	**LAMA2 probe** 14990-L16726		**Exon 8**
480	Reference probe 08614-L08626	12p	
492	Reference probe 17001-L30500	20q	

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

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.

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. SALSA MLPA Probemix P392-A3 LAMA2 mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*LAMA2*
64-105	Control fragments – see table in probemix content section for more information		
136	Reference probe 19012-L25527	21q	
140	**LAMA2 probe** 14957-L16693		**Exon 65**
148	Reference probe 13226-L14559	1p	
154	**LAMA2 probe** 14924-L16657		**Exon 11**
161	**LAMA2 probe** 14960-L16696		**Exon 33**
166	**LAMA2 probe** 20621-L28934		**Exon 56**
174	**LAMA2 probe** 14961-L18097		**Exon 22**
179	**LAMA2 probe** 14927-L28936		**Exon 56**
184	**LAMA2 probe** 14963-L16699		**Exon 27**
191 Ж	**LAMA2 probe** 14929-SP0232-L18098		**Exon 40**
202 Ж	**LAMA2 probe** 14965-SP0236-L16701		**Exon 53**
208	**LAMA2 probe** 14931-L16664		**Exon 51**
214	**LAMA2 probe** 14932-L16665		**Exon 2**
220	**LAMA2 probe** 14933-L16666		**Exon 26**
228	Reference probe 10244-L26254	10p	
232	**LAMA2 probe** 14969-L16705		**Exon 15**
238	Reference probe 10137-L10599	18q	
247	**LAMA2 probe** 14936-L16669		**Exon 50**
256	**LAMA2 probe** 14937-L16670		**Exon 9**
265 Ж	**LAMA2 probe** 14935-SP0233-L18168		**Exon 39**
274	**LAMA2 probe** 20120-L28935		**Exon 21**
283	**LAMA2 probe** 14974-L16710		**Exon 25**
292	**LAMA2 probe** 14975-L16711		**Exon 47**
301	**LAMA2 probe** 14941-L16674		**Exon 7**
310	Reference probe 13176-L14495	3q	
319	**LAMA2 probe** 14942-L16675		**Exon 58**
328	**LAMA2 probe** 14943-L16676		**Exon 23**
337	**LAMA2 probe** 14944-L16677		**Exon 64**
346	**LAMA2 probe** 14945-L16678		**Exon 36**
355	Reference probe 11614-L12374	12p	
364	**LAMA2 probe** 14946-L16679		**Exon 1**
373 Ж	**LAMA2 probe** 14947-SP0234-L16680		**Exon 35**
384	**LAMA2 probe** 14948-L16681		**Exon 19**
391	**LAMA2 probe** 14949-L16682		**Exon 4**
400	Reference probe 15010-L11786	2p	
409	**LAMA2 probe** 14985-L16721		**Exon 17**
418 Ж	**LAMA2 probe** 14951-SP0235-L16684		**Exon 42**
427	**LAMA2 probe** 14952-L16685		**Exon 63**
436	**LAMA2 probe** 14953-L16686		**Exon 13**
454 Ж	**LAMA2 probe** 14989-SP0240-L16725		**Exon 32**
463	**LAMA2 probe** 14955-L16688		**Exon 29**
475	**LAMA2 probe** 14956-L16689		**Exon 61**
483	Reference probe 11677-L12448	16p	

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

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.

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 3. *LAMA2* probes arranged according to chromosomal location

Length (nt) P391 / P392	SALSA MLPA probe	*LAMA2* exon[a]	Ligation site NM_000426.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*109-111 (Exon 1)*		
256	14972-L16708	Exon 1	72-73	AGCGACTCCTCT-GGCTCCCGAGAA	0.1 kb
364	14946-L16679	Exon 1	210-211	TCACAGGCACAT-CAGCAAAGAGGT	166.6 kb
192	14964-L16700	Exon 2	9 nt before exon 2	TCATTTGTCCAT-CTTTTTCAGGTT	0.1 kb
214	14932-L16665	Exon 2	321-322	AAATTGGTAGAA-CATGTCCCTGGG	9.8 kb
202	14930-L16663	Exon 3	425-426	TATTGATGGAAA-GAACACTTGGTG	38.4 kb
391	14949-L16682	Exon 4	582-583	GAACGCTCTCTT-GATGATGTTGAA	0.1 kb
363	14981-L16717	Exon 4	692-693	TGCCAAAGATGA-TGAGGTCATCTG	45.7 kb
310 Ж	20119-SP0237-L16713	Exon 5	874-875; 903-904	ATGCTGACTTGA-29 nt spanning oligo-ATTGACCCCATT	3.0 kb
208	14966-L16702	Exon 6	994-995	ATGCCAGGGCTT-GTCCACTTGATC	2.0 kb
301	14941-L16674	Exon 7	1090-1091	GATTCCATCAGA-AACCCTGGAGAG	5.5 kb
462	14990-L16726	Exon 8	1219-1220	ATATACGTGGAA-AGTACATTGGAG	11.0 kb
256	14937-L16670	Exon 9	1364-1365	TTGCGATCCAAT-TGGTTCCTTAAA	12.1 kb
142	14922-L16655	Exon 10	1470-1471	GTGAGCTGTGAT-CGGTGTGCCAGG	12.6 kb
154	14924-L16657	Exon 11	1684-1685	GGGTTTCAAACA-GATGTCAGAGTT	2.6 kb
427	14987-L16723	Exon 12	26 nt after exon 12	GCTTCCCGCTAT-TCTGCTTTAACT	57.3 kb
436	14953-L16686	Exon 13	17 nt after exon 13	GTACTGAGAGCA-TGTTCACCCGTG	2.0 kb
172	14926-L16659	Exon 14	2141-2142	TATGACAGTGCT-TGCGAATTTGAA	8.6 kb
232	14969-L16705	Exon 15	2292-2293	CAGTGCCCACCA-GGGTATACTGGC	6.3 kb
346	14980-L16716	Exon 16	2356-2357	ACGGCACTATTT-TTGGTGGCATCT	3.6 kb
409	14985-L16721	Exon 17	2513-2514	AGGAACCTCTGA-AGACTGTCAACC	17.3 kb
	No probe	Exon 18			
384	14948-L16681	Exon 19	2771-2772	CTCCTGTCTGAT-ATGTAAACCAGG	3.7 kb
384	14983-L16719	Exon 20	2962-2963	GTGACAAATGCA-AGGTAAGGAGTA	6.1 kb
274	20120-L28935	Exon 21	3121-3122	ACGGCTATTTCA-ACTTCCAAGAAG	2.9 kb
174	14961-L18097	Exon 22	3170-3171	TCATCTGGGTAA-TAATTGTGACCC	12.2 kb
328	14943-L16676	Exon 23	3364-3365	ATCCAAAATTCT-CTGGTGCAAAAT	1.8 kb
214	14967-L28797	Exon 24	3585-3586	GATGCCAAGAAT-CCACTTGGCTGC	0.9 kb
283	14974-L16710	Exon 25	3785-3786	CCTGATGAGAGA-AGATCTCCATTT	0.3 kb
220	14933-L16666	Exon 26	3977-3978	TATCGTCAGGCA-TATGGCTGCTCC	0.2 kb
184	14963-L16699	Exon 27	4079-4080	CCATAGAACTGT-GACCCGAGAAGA	4.5 kb
328 Ж	14978-SP0238-L16714	Exon 28	4214-4215; 4241-4242	ACGTGGAACAAC-27 nt spanning oligo-AAAATGTGATTG	7.8 kb
463	14955-L16688	Exon 29	4375-4376	CATGTCAATGTA-ATGGACACAGCA	14.0 kb
238	14970-L16706	Exon 30	4471-4472	GTGCTCTTGGAT-ACTATGGAATTG	7.0 kb
409	14950-L16683	Exon 31	4618-4619	GATATGAAGGCC-AGTACTGTGAAA	3.9 kb
454 Ж	14989-SP0240-L16725	Exon 32	4708-4709; 4735-4736	ATCCCTATGGCT-27 nt spanning oligo-CAGGATTCTGCA	13.0 kb
161	14960-L16696	Exon 33	4852-4853	GCACTGGCCTTC-TTCTCGGTGACT	3.7 kb
416	14986-L16722	Exon 34	5008-5009	AGAGGCTTATTC-AGCTGGCAGAGG	13.3 kb
373 Ж	14947-SP0234-L16680	Exon 35	5133-5134; 5163-5164	AACACAAGAGCA-30 nt spanning oligo-GCCCGGGATGCA	8.4 kb
346	14945-L16678	Exon 36	5245-5246	CCTTTGAGAGAA-ATTTGGAAGGGC	1.7 kb
373	14982-L16718	Exon 37	5522-5523	TCGCCTATTTGC-AGTAAATCAGAA	8.1 kb
283	14939-L16672	Exon 38	5668-5669	ACTCCATCATAG-ACGTGAGTATTG	1.1 kb
265 Ж	14935-SP0233-L18168	Exon 39	5757-5758; 5784-5785	GAAATAAAGGAC-27 nt spanning oligo-GCTGAGAGCCAC	1.4 kb
191 Ж	14929-SP0232-L18098	Exon 40	5868-5869; 5895-5896	ATCTCCTTCAAT-27 nt spanning oligo-AATATTAAGGAC	24.0 kb
274	14938-L16671	Exon 41	6042-6043	TTCAGGATTCTT-AACGAAGCCAAG	10.9 kb
418 Ж	14951-SP0235-L16684	Exon 42	6104-6105; 6137-6138	AAATGGCTTAAA-33 nt spanning oligo-GGATCTCTTGAG	2.3 kb

Length (nt) P391 / P392	SALSA MLPA probe	*LAMA2* exon[a]	Ligation site NM_000426.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
292	14940-L16673	Exon 43	2 nt after exon 43	TCCAAGAACAGT-AAGATCTCCTTT	4.7 kb
	No probe	Exon 44			
231	14934-L16667	Exon 45	6407-6408	TGCCACTGTCAA-AAATTTAGAACA	7.3 kb
184	14928-L16661	Exon 46	6582-6583	ATTCGAACATAC-AAACCAGAAATC	1.2 kb
292	14975-L16711	Exon 47	6792-6791 reverse	ATACGGTACCAA-TATGAGTCATCA	6.0 kb
	No probe	Exon 48			
220	14968-L16704	Exon 49	7011-7012	ACATTCACTGGC-TGCATGGGAGAA	4.1 kb
247	14936-L16669	Exon 50	7156-7157	AAGGTTATGCAT-TGGTCAGCCGTC	0.9 kb
208	14931-L16664	Exon 51	7347-7348	TCCGTTGTCAGC-AATCAAAACCAT	8.0 kb
337 Ж	14979-SP0239-L16715	Exon 52	7477-7478; 7513-7514	CTTCTGGAAACA-36 nt spanning oligo-ATTTTGGTGGCC	2.1 kb
202 Ж	14965-SP0236-L16701	Exon 53	4 nt after exon 53; 37 nt after exon 53	AAAGCAAGGTAA-33 nt spanning oligo-TGGGTTGGGTAA	3.3 kb
178	14962-L16698	Exon 54	7614-7615	ATTGAAATTTCA-AGAACTCCGTAC	2.7 kb
247	14971-L16707	Exon 55	7833-7834	GCACCACCTAGG-AGAAAACGAAGG	5.1 kb
148	14958-L16694	Exon 56	7896-7897	GGCCGTCTGGAA-GTGCATCTCTCC	0.1 kb
166	20621-L28934	Exon 56	7985-7986	AGAACATTCCGT-TCATGTAGAGCG	0.1 kb
179	14927-L28936	Exon 56	58 nt after exon 56	CCTTTGCTAGCA-TCGGTATCTATC	5.3 kb
436	14988-L18028	Exon 57	8047-8048	GAAGATACATGC-AAAACCTGACAG	0.5 kb
319	14942-L16675	Exon 58	8276-8277	AGATGGAGCAGC-TCCAGCTGAAAT	10.3 kb
454	14954-L16687	Exon 59	8382-8383	TCAGAACCAGCT-CTTTTGATAGGG	0.5 kb
160	14925-L18029	Exon 60	8572-8573	TGAGAAATGGAT-TGCCCTACTTCA	2.1 kb
475	14956-L16689	Exon 61	8767-8768	GAATGCTGTATG-TTGGTGGGTTAC	2.3 kb
391	14984-L16720	Exon 62	8915-8916	GACATGTTTTGC-AAATGCTCAGAG	4.9 kb
427	14952-L16685	Exon 63	9048-9049	CTTCTGGGGATC-AGTAGTCAAAAA	2.1 kb
337	14944-L16677	Exon 64	9253-9254	AGGTGGAAGCCC-AAAGCCCAAACC	1.7 kb
140	14957-L16693	Exon 65	9342-9343	CAGTTTGGCCTA-ACAACCAGTATT	0.1 kb
301	14976-L16712	Exon 65	9429-9430	AATTTTGCCAAG-GCCCTGGAACTG	
		stop codon	*9475-9477 (Exon 65)*		

[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.

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.

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.

References

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Selected publications using SALSA MLPA Probemixes P391/P392 LAMA2

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P391 product history	
Version	*Modification*
A3	Two reference probes have been added and one reference probe has been replaced.
A2	Four reference probes have been replaced and one reference probe has been removed. In addition, the control fragments have been adjusted (QDX2).
A1	First release.

P392 product history	
Version	*Modification*
A3	Two reference probes have been replaced and one probe length has been adjusted.
A2	Four reference probes have been replaced and the control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description

Version A3/A3-02 – 15 August 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *LAMA2* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 3 in order to better reflect the true lengths of the amplification products.
- Modifications in the P391-A2 and P392-A2 product versions corrected in the product history tables.

Version A3/A3-01– 25 February 2019 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version.
- Small changes in Table 1 and Table 2.

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