Product Description SALSA® MLPA® Probemix P048-C3 LMNA/MYOT/ZMPSTE24

To be used with the MLPA General Protocol.

Version C3. As compared to version C2, three reference probes have been replaced and one probe has been adjusted in length. For complete product history see page 8.

Catalogue numbers:
- **P048-025R:** SALSA MLPA Probemix P048 LMNA/MYOT/ZMPSTE24, 25 reactions.
- **P048-050R:** SALSA MLPA Probemix P048 LMNA/MYOT/ZMPSTE24, 50 reactions.
- **P048-100R:** SALSA MLPA Probemix P048 LMNA/MYOT/ZMPSTE24, 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 P048-C3 LMNA/MYOT/ZMPSTE24 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *LMNA*, *MYOT, CAV3* and *ZMPSTE24* genes, which is associated with Laminopathies.

Laminopathies have emerged as clinically heterogeneous genetic disorders due to mutations in lamins or lamin-associated proteins. Lamins are structural protein components of the nuclear lamina, a protein network underlying the inner nuclear membrane that determines nuclear shape and size. The lamins constitute a class of intermediate filaments. Three types of lamins, A, B, and C, have been described in mammalian cells.

Laminopathies regroup at least eight distinct diseases, belonging to the groups of skeletal and/or cardiac muscular dystrophies, axonal neuropathies, premature ageing syndromes and familial lipodystrophies. These diseases, such as Emery-Dreifuss muscular dystrophy, Hutchinson-Gilford progeria syndrome and limb-girdle muscular dystrophy type 1B (LGMD1B), result from alterations in the *LMNA* gene, encoding type A-lamins.

Pathophysiological mechanisms explaining how mutations in a unique gene could lead to such various phenotypes are still unknown, but probably involve alterations in cellular mechanical stress responses, in gene expression, and/or in post-translational maturation of lamin A. One gene that is involved in the post-translational processing of lamin A precursor is *ZMPSTE24* (also known as *FACE1* in human). Loss of function of the *ZMPSTE24* gene and accumulation of precursor lamin A has been correlated with restrictive dermopathy (RD).

Mutations in the *MYOT* gene are associated with LGMD1A and myofibrillar myopathies. Mutations in the *CAV3* gene are associated with LGMD1C.

The *LMNA* gene (12 exons), spans ~25.4 kb of genomic DNA and is located on 1q22, ~156 Mb from the p-telomere. The *ZMPSTE24* gene (10 exons), spans ~36 kb of genomic DNA and is located on 1p34.2, ~40 Mb from the p-telomere. The *MYOT* gene (10 exons), spans ~20 kb of genomic DNA and is located on chromosome 5q31.2, ~138 Mb from the p-telomere. The *CAV3* gene (2 exons), spans ~13 kb of genomic DNA and is located on chromosome 3p25.3, ~8.8 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK304462/.

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 *LMNA*, *MYOT, CAV3* and *ZMPSTE24* exon numbering used in this P048-C3 LMNA/MYOT/ZMPSTE24 product description are: the exon numbering from the RefSeq transcript NM_170707.2, NM_006790.3, NM_033337.3 and NM_005857.5, which is identical to the LRG_254, LRG_201, LRG_329 and LRG_212 and NG_008692.2, NG_006790.2, NG_008797.2 and NG_005857.3 sequences, respectively. The exon numbering and NM_ sequence used have been retrieved on 08/2020. 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 P048-C3 LMNA/MYOT/ZMPSTE24 contains 44 MLPA probes with amplification products between 131 and 463 nucleotides (nt). The P048-C3 probemix contains probes for all 12 exons of the *LMNA* gene. Two probes are present for exon 1. This probemix furthermore contains probes for all exons of the *ZMPSTE24*, *MYOT* and *CAV3* genes. 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 Laminopathies. 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 *LMNA*, *MYOT*, *CAV3* and *ZMPSTE24* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P048 LMNA/MYOT/ZMPSTE24.
- 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.

LMNA/MYOT/ZMPSTE24/CAV3 mutation database: https://databases.lovd.nl/shared/genes/LMNA. 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 *LMNA* exons 1 and 3 but not exon 2) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P048-C3 LMNA/MYOT/ZMPSTE24

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	LMNA	ZMPSTE24	MYOT	CAV3
64-105	Control fragments – see table in probemix content section for more information					
131 *	Reference probe 22089-L15549	17q24				
137	**ZMPSTE24 probe** 15689-L21730			**Exon 7**		
148	**ZMPSTE24 probe** 15691-L17658			**Exon 10**		
154	**LMNA probe** 11552-L12299		**Exon 5**			
160	**LMNA probe** 11553-L12300		**Exon 9**			
166	**MYOT probe** 11554-L12301				**Exon 4**	
172	**MYOT probe** 11555-L12302				**Exon 1**	
179	**ZMPSTE24 probe** 15687-L18494			**Exon 2**		
184	**MYOT probe** 11556-L12303				**Exon 8**	
190	**MYOT probe** 11557-L12304				**Exon 5**	
196	Reference probe 10138-L10600	18q11				
202 ¥	**ZMPSTE24 probe** 20983-L32311			**Exon 9**		
208	**ZMPSTE24 probe** 15684-L18495			**Exon 1**		
214	**MYOT probe** 11559-L13505				**Exon 3**	
220	**LMNA probe** 05939-L05368		**Exon 12**			
230	Reference probe 01783-L01347	13q14				
238	**CAV3 probe** 09681-L10061					**Exon 1**
247	**LMNA probe** 02478-L01971		**Exon 11**			
256	Reference probe 10702-L11284	6p12				
262	**MYOT probe** 20981-L13504				**Exon 9**	
267 *	Reference probe 14758-L32312	9q32				
274	**CAV3 probe** 17349-L21793					**Exon 2**
280	**LMNA probe** 12287-L13799		**Exon 1**			
286	**LMNA probe** 12027-L13939		**Exon 1**			
292	**MYOT probe** 11560-L12307				**Exon 7**	
301	**LMNA probe** 01916-L01460		**Exon 2**			
310	**ZMPSTE24 probe** 16705-L19884			**Exon 3**		
315	**LMNA probe** 15694-L20323		**Exon 7**			
320	Reference probe 05802-L20124	15q15				
330	**LMNA probe** 01918-L21732		**Exon 4**			
337	**LMNA probe** 01919-L01463		**Exon 6**			
346	**ZMPSTE24 probe** 15692-L17659			**Exon 4**		
355 Ж	**ZMPSTE24 probe** 16895-SP0379-L19286			**Exon 5**		
361	**LMNA probe** 13216-L14549		**Exon 8**			
370	**ZMPSTE24 probe** 20982-L17657			**Exon 8**		
382	**LMNA probe** 01922-L01466		**Exon 10a**			
391	**LMNA probe** 15693-L17660		**Exon 3**			
400 *	Reference probe 14204-L15818	11p15				
412	**MYOT probe** 11561-L21731				**Exon 6**	
418	**MYOT probe** 11562-L12309				**Exon 2**	
427	**MYOT probe** 11563-L14166				**Exon 10**	
436	Reference probe 09614-L09909	20p12				
454	**ZMPSTE24 probe** 15695-L17662			**Exon 6**		
463	Reference probe 10108-L10532	8q22				

* New in version C3 (from lot C3-0620 onwards).
¥ Changed in version C3 (from lot C3-0620 onwards). Minor alteration, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites.

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

Table 2. P048 probes arranged according to chromosomal location

Table 2a. *ZMPSTE24* gene

Length (nt)	SALSA MLPA probe	*ZMPSTE24* Exon[a]	Ligation site NM_005857.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*37-39 (exon 1)*		
208	15684-L18495	Exon 1	12-13	GGTGCACGCTGA-AGGAGCCGGCGG	2.6 kb
179	15687-L18494	Exon 2	200-201	ACCACCGGAGTT-AGGACAGATCAT	6.9 kb
310	16705-L19884	Exon 3	348-349	CTCTGGAGACTT-TCTGGACGGTTC	0.7 kb
346	15692-L17659	Exon 4	478-479	ATAATACTTTTG-TGATAGAAGAAA	1.5 kb
355 Ж	16895-SP0379-L19286	Exon 5	16 nt before exon 5; 525-526	TTCTTGTGGTAA-31 nt spanning oligo-ATGAAAGATGCA	2.0 kb
454	15695-L17662	Exon 6	725-726	CACACCTCTGCC-TGAGGGAAAGCT	9.4 kb
137	15689-L21730	Exon 7	836-837	CAGCAATGCTTA-TTTTTATGGCTT	4.6 kb
370	20982-L17657	Exon 8	1040-1041	TGTACTAGGCCA-TGAACTGGGGCA	5.0 kb
202 ¥	20983-L32311	Exon 9	1181-1182	TTATGATAGCCA-ACCCACTCTTAT	3.0 kb
148	15691-L17658	Exon 10	2703-2704	AAAGTGGGATCA-ACTGTACGCCTT	
		Stop Codon	*1462-1464 (exon 10)*		

¥ Changed in version C3 (from lot C3-0620 onwards). Minor alteration, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites.

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.

Table 2b. *LMNA* gene

Length (nt)	SALSA MLPA probe	*LMNA* Exon[a]	Ligation site NM_170707.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*250-252 (exon 1)*		
280	12287-L13799	Exon 1	534-535	CTTGACTCAGTA-GCCAAGGAGCGC	0.1 kb
286	12027-L13939	Exon 1	589-590	GTGAGGAGTTTA-AGGAGCTGAAAG	15.4 kb
301	01916-L01460	Exon 2	639-640	CTGATAGCTGCT-CAGGCTCGGCTG	3.8 kb
391	15693-L17660	Exon 3	781-780 reverse	CTTCTTGGCCTC-ACCTAGGGCTGC	0.4 kb
330	01918-L21732	Exon 4	936-937	CTGGTGGAGATT-GACAATGGGAAG	0.3 kb
154	11552-L12299	Exon 5	1067-1066 reverse	ACTGCCTGGCAT-TGTCCAGCTGGA	0.9 kb
337 #	01919-L01463	Exon 6	1335-1336	TACCAGGAGCTT-CTGGACATCAAG	0.3 kb
315	15694-L20323	Exon 7	1515-1516	CGCAAACTGGAG-TCCACTGAGAGC	0.4 kb
361	13216-L14549	Exon 8	161 nt before exon 8	TGTGTCCACAGA-TCATGGCTATTA	0.4 kb
160	11553-L12300	Exon 9	1831-1830 reverse	GATGAGAGCCGT-ACGCAGGCTGTT	0.5 kb
382	01922-L01466	Exon 10a	1893-1894	CGCTCAGTGACT-GTGGTTGAGGAC	0.9 kb
247	02478-L01971	Exon 11	2094-2095	TCCTCTGGCTCT-TCTGCCTCCAGT	0.4 kb
220	05939-L05368	Exon 12	9 nt before exon 12	CTCCTCTGTTTT-CTCTCTTAGAGC	
		Stop Codon	*2242-2244 (exon 12)*		

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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.

Table 2c. *CAV3* gene

Length (nt)	SALSA MLPA probe	*CAV3* Exon[a]	Ligation site NM_033337.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*78-80 (exon 1)*		
238	09681-L10061	Exon 1	142-143	CTGCAAGGAGAT-TGACCTGGTGAA	11.8 kb
274	17349-L21793	Exon 2	435-436	GCATCAGCCACA-TCTACTCACTCT	
		Stop Codon	*531-533 (exon 2)*		

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.

Table 2d. *MYOT* gene

Length (nt)	SALSA MLPA probe	*MYOT* Exon[a]	Ligation site NM_006790.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*310-312 (exon 2)*		
172	11555-L12302	Exon 1	14 nt after exon 1	AAGTCCCTTCTT-TATTCCTACTAC	2.5 kb
418	11562-L12309	Exon 2	164-165	TCAGGATCTCAA-CAAGGAAGAGCA	5.4 kb
214	11559-L13505	Exon 3	726-727	ACTGCAAATGCT-AAGCCCATACCA	1.7 kb
166	11554-L12301	Exon 4	889-890	TAGGACCACAGA-ATGCAGCTGCTG	3.2 kb
190	11557-L12304	Exon 5	957-956 reverse	AGTCGCGCATGT-TCTGAGTTGTGT	1.2 kb
412	11561-L21731	Exon 6	1063-1064	ACCCACCACGTT-TCATTCAAGTGC	1.4 kb
292	11560-L12307	Exon 7	1163-1164	GTCATGGTATCT-AAATGGAAGAAC	2.7 kb
184	11556-L12303	Exon 8	1431-1432	CAGATCTCGGCT-ATACCTCCACCA	0.8 kb
262	20981-L13504	Exon 9	1611-1612	GTGACTACATGT-AACACAAGATTA	0.3 kb
427	11563-L14166	Exon 10	1686-1687	GTTCGACCAACA-TTCAGCAAATAT	
		Stop Codon	*1804-1806 (exon 10)*		

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.

Related SALSA MLPA probemixes

- P034/P035 DMD: Duchenne muscular dystrophy and Becker muscular dystrophy. Contain probes for all DMD exons.
- P061 Lissencephaly: Contains probes for the *POMT1* gene, involved in LGMD2K.
- P116 SGC: Contains probes for the *SGCA*, *SGCB*, *SGCD*, *SGCG* and *FKRP* genes, involved in LGMDs.
- P176 CAPN3: Contains probes for the *CAPN3* gene, involved in LGMD2A.
- P268 DYSF: Contains probes for the *DYSF* gene, involved in LGMD2B.
- P436 ANO5: Contains probes for the *ANO5* gene, involved in LGMD2L.

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 P048 LMNA/MYOT/ZMPSTE24

- Haye D et al. (2016) Failure of ossification of the occipital bone in mandibuloacral dysplasia type B. *Am J of Med Gen.* 170: 2750-2755.
- Van Spaendonck-Zwarts K et al. (2013). Genetic analysis in 418 index patients with idiopathic dilated cardiomyopathy: overview of 10 years' experience. *Eur J Heart Fail.* 15:628–636.
- Weterings A et al. (2013). A novel lamin A/C mutation in a Dutch family with premature atherosclerosis. *Atherosclerosis.* 229:169-173.
- Norton N et al. (2011). Assessment of LMNA copy number variation in 58 probands with dilated cardiomyopathy. *Clin Transl Sci.* 4:351–352.
- Marsman R et al (2011). A complex double deletion in LMNA underlies progressive cardiac conduction disease, atrial arrhythmias, and sudden death. *Circ Cardiovasc Genet.* 4:280-287.

P048 Product history	
Version	*Modification*
C3	Three reference probes have been replaced and one probe length has been adjusted.
C2	Several probes have been adjusted in length.
C1	10 target probes for the gene ZMPSTE24 have been included. The LMNA probe for exon 8 and exon 3, the CAV3 probe for exon 2 have been replaced. Two flanking probes have been removed and all reference probes have been replaced.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	The LMNA exon 1 probe has been replaced by two new ones. Probes for LMNA exon 5 and 9 have been added. Probes for all 10 exons of the MYOT gene have been included. Finally extra control fragments at 88-96-100-105 nt have been added.
A1	Fist release

Implemented changes in the product description

Version C3-01 — 05 October 2020 (02P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *ZMPSTE24 gene* is updated according to the NM_005857.5 sequence.
- Warning added to Table 2b for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Added P034/P035 DMD in Related SALSA MLPA probemixes.

Version 18 – 21 February 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).
- New reference added at page 2.

Version 17 – 01 June 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Warning on probe located in CpG island removed.
- Various minor textual changes.
- New references added on page 2.

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