Product Description SALSA® MLPA® Probemix P034-B2 DMD-1 & P035-B1 DMD-2

To be used with the MLPA General Protocol.

P034 version B2. As compared to version B1, two probes have a very small change in length, but no change in sequence detected. For complete product history see page 10.

P035 version B1. As compared to version A3, all reference probes and the probes for *DMD* exons 12-15, 19, 32, 33, 39, 40, 56, 71, 78, and 79 have been replaced. For complete product history see page 10.

Catalogue numbers:

- **P034-025R:** SALSA MLPA probemix P034 DMD-1, 25 reactions.
- **P034-050R:** SALSA MLPA probemix P034 DMD-1, 50 reactions.
- **P034-100R:** SALSA MLPA probemix P034 DMD-1, 100 reactions.

- **P035-025R:** SALSA MLPA probemix P035 DMD-2, 25 reactions.
- **P035-050R:** SALSA MLPA probemix P035 DMD-2, 50 reactions.
- **P035-100R:** SALSA MLPA probemix P035 DMD-2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman 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.

Intended use: The SALSA MLPA probemixes P034 DMD-1 and P035 DMD-2 are an in vitro diagnostic (IVD)[1] or research use only (RUO) assay for the detection of exon deletions or duplications in the human *DMD* gene as a cause for Duchenne muscular dystrophy and/or Becker muscular dystrophy and for carrier screening thereof. This assay can be used with human DNA derived from peripheral blood, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or fetal blood.

In the majority of patients, most defects in the *DMD* gene are copy number variations (CNVs), however point mutations can occur which will not be detected by MLPA. It is therefore recommended to use these SALSA MLPA probemixes in combination with sequence analysis. Copy number changes detected by only a single probe always require validation by another method. These probemixes are not intended to be used as standalone assays for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that these probemixes are for In Vitro Diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Germline defects in the dystrophin *(DMD)* gene are the most frequent cause of Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). DMD and BMD occur almost exclusively in males as they are inherited in an X-linked recessive manner. DMD usually has an early onset in childhood with delayed milestones, which include delays in sitting and standing independently. Proximal weakness causes a waddling gait and difficulty in climbing. DMD is rapidly progressive, with affected children being wheelchair dependent by the age of 13. Respiratory complications and cardiomyopathy occur in individuals with DMD after the age of 18 and a few survive beyond the third decade of life. In contrast, BMD has a slower rate of progression and patients on average survive until their mid-40s. More information on both conditions is available at http://www.ncbi.nlm.nih.gov/books/NBK1119/.

Deletions and duplications of complete exons in the *DMD* gene are the most frequent cause of DMD/BMD and are usually missed by standard sequence analysis. Most of these deletions and duplications can be detected by the MLPA technique and hence MLPA complements sequence analysis of the *DMD* gene. The frequency of deletions/duplications in the *DMD* gene in DMD/BMD patients has been estimated at 60-70% for deletions and 5-10% for duplications (http://www.ncbi.nlm.nih.gov/books/NBK1119/). Best practice guidelines on molecular diagnostics in Duchenne/Becker muscular dystrophies have been published by Abbs S. et al (2010).

Gene structure: The *DMD* gene spans ~2100 kilobases (kb) on chromosome Xp21.1-p21.2. The *DMD* LRG_199 is identical to the NCBI NG_012232. The LRG sequence is available at https://www.lrg-sequence.org/.

Transcript variants: http://www.ncbi.nlm.nih.gov/gene/1756 describes several transcript variants of the *DMD* gene.
The NM_004006.2 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_004006.2, represents the transcript variant in muscle, Dp427m. This sequence is a reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 1 (245-247) and the stop codon is located in exon 79 (11300-11302).
In addition, the NM_000109.3 sequence, see https://www.ncbi.nlm.nih.gov/nuccore/NM_000109.3, represents the transcript variant Dp427c which is predominantly expressed in the brain. This transcript uses an alternative promoter/exon 1, which is located upstream of the Dp427m transcript promoter. The other 78 exons are identical in both transcripts. The ATG translation start site of the Dp427c transcript variant is located in exon 1 (345-347).

Exon numbering: The exon numbering used in this P034-B2 DMD-1 and P035-B1 DMD-2 product description is the exon numbering from the RefSeq transcript NM_004006.2, which is identical to the LRG_199 sequence. The exon numbering and NM sequence used are from 10/2018, but can be changed (by NCBI) after the release of the product description.

P034-B2 DMD-1 and P035-B1 DMD-2 probemixes content: The P034 and P035 probemixes contain together one probe for each of the 79 exons of the *DMD* gene transcript variant Dp427m. In addition, one probe is present in P035-B1 for the alternative promoter/exon 1 found in transcript variant Dp427c of *DMD*. Performing two MLPA reactions is thus sufficient to investigate the copy number of all exons.

The P034-B2 probemix contains 49 MLPA probes with amplification products between 130 and 500 nt in length including nine reference probes. The P035-B1 probemix contains 48 MLPA probes with amplification products between 130 and 500 nt in length, eight of which are reference probes. The identity of the genes detected by the reference probes is 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 and 96 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 of the same sex 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 from human peripheral blood, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or fetal blood, 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: 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 DMD or BMD. It is recommended to use reference and patient samples of the same sex to facilitate interpretation. 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 Biobank (https://catalog.coriell.org) and 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. Sample ID numbers NA05117 (female), NA05123 (male), NA23087 (female), NA23094 (female) and NA10283 (male) from the Coriell Institute have been tested at MRC-Holland and can be used as positive control samples to detect a heterozygous exon 45 deletion (only suitable for P034), an exon 45-62 duplication, a heterozygous exon 2-30 duplication, a heterozygous exon 35-43 deletion, and an exon 72-79 deletion (only suitable for P035), respectively. The quality of cell lines can change, therefore samples should be validated before use.

Performance characteristics: The expected number of *DMD* deletions/duplications that can be detected with these MLPA probemixes is approximately 70% of all *DMD* mutations in most patient populations.

The analytical sensitivity and specificity for the detection of deletions/duplications in Duchenne and Becker muscular dystrophy patients (based on a 2010-2018 literature review) is very high and can be considered >99%. Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For P034 and P035, the latest versions 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 expected results for the *DMD* region specific MLPA probes in female DNA samples are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication) and occasionally 4 (heterozygous triplication). For male DNA samples, copy numbers of 1 (normal), 0 (deletion), or 2 (duplication) can be expected.

The standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes 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:

Copy Number status **Male samples vs Male reference samples**	**Dosage quotient**
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Duplication	1.65 < DQ < 2.25
Ambiguous copy number	All other values

Copy Number status **Female samples vs Female reference samples**	**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 or in or near the *DMD* gene. 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 (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 in some cases not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Notes DMD results:

- DMD/BMD is an X-linked recessive disorder. In males, alteration of the single *DMD* copy due to a mutation, deletion or partial duplication is therefore sufficient to cause the condition. In females, inactivation of a single copy of the gene is not expected to be pathogenic.
- A heterozygous deletion of one or more *DMD* exons that are present in the major transcript variant Dp427m is expected to result in Duchenne or Becker muscular dystrophy in males. Deletion of the promoter/exon 1 of this transcript variant, which is the most abundant transcript in muscle, has been reported to result in X-linked dilated cardiomyopathy (Muntoni F. et al. 1993). While such a deletion theoretically should preclude muscle-specific transcription, relatively high levels of dystrophin were still detected. The authors suggest that transcription was driven by the alternative brain promoter, present in exon 1 of transcript Dp427c and not in Dp427m, leading to a different phenotype.
- A duplication of an internal part of a gene usually results in a defective copy of that gene, as the duplicated sequence is typically located directly adjacent to the original sequence, resulting in a defective transcript. Duplication of the complete *DMD* gene is not expected to result in disease. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might in some cases not result in inactivation of that gene copy.
- Please note that Schwartz et al. (2007) have reported a completely healthy adult male with a deletion of exon 16 and part of introns 15 and 16. Their findings suggest that some gene re-arrangements of the

dystrophin gene may not always be disease-causing. Please be cautious with the diagnosis of dystrophinopathy in cases of single exon in-frame deletions.
- Given the above-mentioned findings, one should be cautious with the prediction of an expected phenotype based on genotype. Factors like in-frame/out of-frame, extent and location of mutations in the *DMD* gene have different influences on the phenotype. The www.DMD.nl website has a tool to predict the effect of exons deletions/duplications on the reading frame.

Limitations of the procedure:
- The SALSA MLPA probemixes P034 DMD-1 and P035 DMD-2 will not detect point mutations in the *DMD* gene, which are the second most common cause of genetic defects in the *DMD* gene.
- 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.
- For use on (un)cultured amniocytes, contamination of the sample with maternal DNA may lead to wrong conclusions.
- For use on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and foetus have been described due to maternal contamination, postzygotic nondisjunction, postzygotic isochromosome formation, mosaic situations, and complications in DNA sampling in twin pregnancies (Van den Berg et al. 2006).
- 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. In males, obtaining a PCR amplicon of correct size for that particular exon indicates that a false positive result may have been obtained. Finding a heterozygous mutation or polymorphism in female samples 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 one or 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.

***DMD* mutation database:** http://www.DMD.nl. We strongly encourage users to deposit positive results in the *DMD* Mutation Database. 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 *DMD* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P034-B2 DMD-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a] Reference	*DMD*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 13499-L02104	Xp11	
142	**DMD probe** 01353-L25384		**Exon 1 of Dp427m**
149	**DMD probe** 18831-L25385		**Exon 41**
157	**DMD probe** 01355-L25386		**Exon 21**
165	**DMD probe** 01356-L25387		**Exon 61**
172	**DMD probe** 01357-L25388		**Exon 2**
178	Reference probe 07655-L07361	Xp11	
187	**DMD probe** 01711-L25389		**Exon 42**
193	**DMD probe** 18998-L24802		**Exon 22**
199	**DMD probe** 01897-L25390		**Exon 62**
205	Reference probe 07672-L26039	Xp22	
213	**DMD probe** 01361-L25391		**Exon 3**
220	**DMD probe** 18221-L25392		**Exon 43**
226	**DMD probe** 19138-L25106		**Exon 23**
231	Reference probe 00821-L09487	Xp22	
241	**DMD probe** 01364-L25393		**Exon 63**
247	**DMD probe** 01365-L25394		**Exon 4**
254	**DMD probe** 01366-L25396		**Exon 44**
262 Δ	**DMD probe** 01958-L25397		**Exon 24**
268	**DMD probe** 01368-L25398		**Exon 64**
278	Reference probe 05893-L08952	Xq22	
284	**DMD probe** 01954-L25724		**Exon 5**
291	**DMD probe** 01370-L01287		**Exon 45**
303	**DMD probe** 01371-L25399		**Exon 25**
312	**DMD probe** 19002-L26038		**Exon 65**
319	**DMD probe** 01373-L25725		**Exon 6**
325	**DMD probe** 01374-L25401		**Exon 46**
332	**DMD probe** 01375-L25402		**Exon 26**
341	**DMD probe** 01376-L25403		**Exon 66**
346	Reference probe 03149-L14468	Xq21	
357	**DMD probe** 01713-L25404		**Exon 7**
364	**DMD probe** 01378-L25405		**Exon 47**
373	**DMD probe** 01379-L25406		**Exon 27**
381	**DMD probe** 01960-L25407		**Exon 67**
391	**DMD probe** 19004-L24808		**Exon 8**
398	**DMD probe** 01382-L25408		**Exon 48**
405	**DMD probe** 01716-L25410		**Exon 28**
413 ¥	**DMD probe** 02482-L28035		**Exon 68**
420	Reference probe 00820-L25090	Xq26	
427	**DMD probe** 01385-L25412		**Exon 9**
436	**DMD probe** 19006-L24810		**Exon 49**
445	**DMD probe** 01387-L25413		**Exon 29**
454	**DMD probe** 19341-L25594		**Exon 69**
461	Reference probe 05632-L26218	Xq28	
469	**DMD probe** 01718-L26101		**Exon 10**
476	**DMD probe** 19340-L25774		**Exon 50**
483	**DMD probe** 19008-L24812		**Exon 30**
493 ¥	**DMD probe** 01392-L27986		**Exon 70**
500	Reference probe 10764-L25900	Xq23	

(a) The exon numbering used in this P034-B2 DMD-1 and P035-B1 DMD-2 product description is the exon numbering from the RefSeq transcript NM_004006.2, which is identical to the LRG_199 sequence. The exon numbering and NM sequence used are from 10/2018, but can be changed (by NCBI) after the release of the product description.

Δ More variable. This probe is sensitive to experimental conditions. Aberrant results should be treated with caution.
¥ Changed in version B2 (from lot B2-1014 onwards). Small change in length, no change in sequence detected.

Table 1b. SALSA MLPA Probemix P035-B1 DMD-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a] Reference	*DMD*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 13498-L06679	Xp22	
141	**DMD probe** 01393-L25414		**Exon 11**
148	**DMD probe** 01394-L25415		**Exon 51**
157	**DMD probe** 01395-L25416		**Exon 31**
166 Ж	**DMD probe** 18996-SP0733-L25739		**Exon 71**
171 Ж	**DMD probe** 18997-SP0734-L25740		**Exon 12**
177	Reference probe 03110-L22383	Xq21	
187	**DMD probe** 02059-L25417		**Exon 52**
193	**DMD probe** 18999-L24803		**Exon 32**
199	**DMD probe** 01949-L25418		**Exon 72**
211	**DMD probe** 19001-L24805		**Exon 13**
219	**DMD probe** 01892-L01050		**Exon 53**
226	**DMD probe** 19140-L25108		**Exon 33**
232	Reference probe 07669-L07375	Xp22	
239	**DMD probe** 01893-L25419		**Exon 73**
247	**DMD probe** 19338-L25591		**Exon 14**
254	**DMD probe** 01894-L25421		**Exon 54**
263	**DMD probe** 01901-L25422		**Exon 34**
269	**DMD probe** 01902-L25423		**Exon 74**
276	Reference probe 02900-L26167	Xq26	
283	**DMD probe** 19141-L25730		**Exon 15**
291	**DMD probe** 01411-L01058		**Exon 55**
297	Reference probe 06476-L26037	Xp22	
303	**DMD probe** 15720-L25424		**Exon 35**
310	**DMD probe** 01413-L25425		**Exon 75**
319	**DMD probe** 02060-L25426		**Exon 16**
325 Ж	**DMD probe** 19003-SP0735-L24807		**Exon 56**
332	**DMD probe** 01416-L25427		**Exon 36**
342	**DMD probe** 01417-L25428		**Exon 76**
350	Reference probe 08245-L09531	Xq26	
358	**DMD probe** 01952-L25429		**Exon 17**
364	**DMD probe** 01419-L25430		**Exon 57**
372	**DMD probe** 03038-L26168		**Exon 37**
381	**DMD probe** 01421-L25432		**Exon 77**
388	**DMD probe** 01891-L25433		**Exon 18**
396 ±	**DMD probe** 01423-L01070		**Exon 58**
407	**DMD probe** 19372-L25772		**Exon 38**
413	**DMD probe** 19342-L25595		**Exon 78**
422	Reference probe 06187-L26204	Xq13	
427	**DMD probe** 19005-L24809		**Exon 19**
436	**DMD probe** 01427-L25436		**Exon 59**
445	**DMD probe** 19007-L24811		**Exon 39**
453	**DMD probe** 19142-L25437		**Exon 79**
466	**DMD probe** 01430-L25438		**Exon 20**
472	**DMD probe** 01431-L25439		**Exon 60**
481	**DMD probe** 19009-L24813		**Exon 40**
490	**DMD probe** 01433-L25440		**Exon 1 of Dp427c**
500	Reference probe 10764-L25900	Xq23	

(a) The exon numbering used in this P034-B2 DMD-1 and P035-B1 DMD-2 product description is the exon numbering from the RefSeq transcript NM_004006.2, which is identical to the LRG_199 sequence. The exon numbering and NM sequence used are from 10/2018, but can be changed (by NCBI) after the release of the product description.

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.
± SNP rs398124074 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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

Length (nt) P034/P035	SALSA MLPA probe	*DMD* Exon[a]	Ligation site[b] NM_004006.2	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*245-247 (Exon 1)*		
490	01433-L25440	Exon 1 of Dp427c	NM_000109.3; 233-234	GGCAGTAATAGA-ATGCTTTCAGGA	127.9 kb
142	01353-L25384	Exon 1 of DP427m	62-63	TTCCCCCTACAG-GACTCAGATCTG	191.3 kb
172	01357-L25388	Exon 2	302-303	TTCAAAAGAAAA-CATTCACAAAAT	170.4 kb
213	01361-L25391	Exon 3	371-372	ACCTCTTCAGTG-ACCTACAGGATG	5.0 kb
247	01365-L25394	Exon 4	471-472	TGCCCTGAACAA-TGTCAACAAGGC	21.5 kb
284	01954-L25724	Exon 5	553-554	GTAGATGGAAAT-CATAAACTGACT	6.7 kb
319	01373-L25725	Exon 6	654-655	AACCAACAGTGA-AAAGATTCTCCT	7.0 kb
357	01713-L25404	Exon 7	801-802	CTGGAATAGTGT-GGTTTGCCAGCA	110.5 kb
391	19004-L24808	Exon 8	10nt after exon 8	AGGTAAAGTGTG-TAAAGGACAGCT	1.1 kb
427	01385-L25412	Exon 9	1102-1103	GCACAGGGATAT-GAGAGAACTTCT	52.8 kb
469	01718-L26101	Exon 10	1231-1232	GAAGACAAGTCA-TTTGGCAGTTCA	0.8 kb
141	01393-L25414	Exon 11	1418-1419	ATTTGACAGCCC-ATCAGGGCCGGG	29.9 kb
171 Ж	18997-SP0734-L25740	Exon 12	1610-1611; 1646-1647	TCCAGAATCAGA-36nt spanning oligo-AAGAAAGAACAA	18.5 kb
211	19001-L24805	Exon 13	1846-1845 reverse	AATAATCTGACC-TTAAGTTGTTCT	22.0 kb
247	19338-L25591	Exon 14	7nt before exon 14	TTGATTGTCTCT-TCTCCAGGTATT	0.2 kb
283	19141-L25730	Exon 15	2010-2011	TCACACAACTGG-CTTTAAAGATCA	7.8 kb
319	02060-L25426	Exon 16	2095-2096	AAGCAATCCATG-GGCAAACTGTAT	20.6 kb
358	01952-L25429	Exon 17	2328-2329	GGAACAGATCCT-GGTAAAGCATGC	27.2 kb
388	01891-L25433	Exon 18	2469-2470	AGAAGCTGTGTT-GCAGAGTCCTGA	16.3 kb
427	19005-L24809	Exon 19	9nt before exon 19	ATCTTTGCTCTC-ATGCTGCAGGCC	10.4 kb
466	01430-L25438	Exon 20	2686-2687	CGGTGGATCGAA-TTCTGCCAGTTG	6.4 kb
157	01355-L25386	Exon 21	2952-2953	GAAAGGACAAGG-ACCCATGTTCCT	12.7 kb
193	18998-L24802	Exon 22	3166-3167	GACTATGAAATC-ATGGAGCAGAGA	3.6 kb
226	19138-L25106	Exon 23	3400-3401	AAACTCCGAAAA-ATTCAGGTAATT	4.0 kb
262 Δ	01958-L25397	Exon 24	3477-3478	ATGGCCTGCCCT-TGGGGATTCAGA	1.1 kb
303	01371-L25399	Exon 25	3590-3591	GGCAGAAGATAA-AGAATGAAGCAG	8.7 kb
332	01375-L25402	Exon 26	3731-3732	CTGTAAGCCTCC-AGAAAGATCTAT	6.2 kb
373	01379-L25406	Exon 27	3907-3908	ACTGAGTCTGTA-AATAGTGTCATA	7.3 kb
405	01716-L25410	Exon 28	4056-4057	TTGGCATGAGTT-ATTGTCATACTT	3.0 kb
445	01387-L25413	Exon 29	4234-4235	GCACAGACCCTA-ACAGATGGCGGA	26.4 kb
483	19008-L24812	Exon 30	4437-4438	AGCTTATATTGC-AGACAAGGTGGA	21.8 kb
157	01395-L25416	Exon 31	4555-4556	AAGGAGGCTGCC-CAAAGAGTCCTG	0.5 kb
193	18999-L24803	Exon 32	4742-4743	AAGTAGTACAGT-CACAGCTAAATC	3.1 kb
226	19140-L25108	Exon 33	4889-4890	TAACAGCTTTGA-AATTGCATTATA	5.8 kb
263	01901-L25422	Exon 34	4983-4984	GCGAAAGGAAAT-GAATGTCTTGAC	15.5 kb
303	15720-L25424	Exon 35	5134-5135	CACCTGAAGAGT-ATCACAGAGGTA	0.5 kb
332	01416-L25427	Exon 36	5324-5325	ACATCACAAAGT-GGATCATTCAGG	1.8 kb
372	03038-L26168	Exon 37	5534-5533 reverse	AATGGCTGCAAA-TCGATGGTTGAG	14.4 kb
407	19372-L25772	Exon 38	5649-5650	GGCTGAAATTCA-GCAGGGGGTGAA	2.4 kb
445	19007-L24811	Exon 39	4nt before exon 39	TGTTGTTTTTGA-TCAGAATGAAGA	2.8 kb
481	19009-L24813	Exon 40	23nt before exon 40	TATTGATATTTT-AATAATGTCTGC	1.0 kb
149	18831-L25385	Exon 41	6055-6056	GAGGGCTTGTCT-GAGGATGGGGCC	32.0 kb
187	01711-L25389	Exon 42	6195-6196	AACGATGATGGT-GATGACTGAAGA	22.6 kb
220	18221-L25392	Exon 43	6439-6440	GCATTGCAAAGT-GCAACGCCTGTG	70.6 kb
254	01366-L25396	Exon 44	6625-6626	GAACAGTTTCTC-AGAAAGACACAA	248.6 kb
291	01370-L01287	Exon 45	6781-6782	ACAGATGCCAGT-ATTCTACAGGAA	36.3 kb
325	01374-L25401	Exon 46	6949-6950	AACATTGCTAGT-ATCCCACTTGAA	2.4 kb
364	01378-L25405	Exon 47	7053-7054	TCTCAAACAATT-AAATGAAACTGG	54.4 kb
398	01382-L25408	Exon 48	7261-7262	CAGTTAAATCAT-CTGCTGCTGTGG	38.5 kb
436	19006-L24810	Exon 49	10nt before exon 49	CTATATGGGTTC-TTTTCCCCAGGA	16.7 kb
476	19340-L25774	Exon 50	13nt after exon 50 reverse	AGAGAATGGGAT-CCAGTATACTTA	46.0 kb

Length (nt) P034/P035	SALSA MLPA probe	*DMD* Exon[a]	Ligation site[b] NM_004006.2	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
148	01394-L25415	Exon 51	7666-7667	GCTCTGGCAGAT-TTCAACCGGGCT	44.4 kb
187	02059-L25417	Exon 52	7873-7872 reverse	CTAGCCTCTTGA-TTGCTGGTCTTG	50.2 kb
219	01892-L01050	Exon 53	8030-8031	CTGAGCAGGTCT-TAGGACAGGCCA	21.4 kb
254	01894-L25421	Exon 54	8152-8153	TGGCAGACAAAT-GTAGATGTGGCA	30.3 kb
291	01411-L01058	Exon 55	8312-8313	AAACTCATAGAT-TACTGCAACAGT	120.5 kb
325 Ж	19003-SP0735-L24807	Exon 56	8627-8628; 27nt after exon 56	AAAAGTCTCTCA-34nt spanning oligo-CACAAATGAATT	10.5 kb
364	01419-L25430	Exon 57	8722-8723	CTGAAAGATGAT-GAATTAAGCCGG	17.8 kb
396 ±	01423-L01070	Exon 58	8853-8854	TGAGACTGTACG-AATATTTCTGAC	0.8 kb
436	01427-L25436	Exon 59	9041-9042	TAGATGAGACCC-TTGAAAGACTCC	33.7 kb
472	01431-L25439	Exon 60	9211-9212	GCGCCTCTGAAA-GAGAACGTGAGC	96.0 kb
165	01356-L25387	Exon 61	9361-9362	AGGCAGCTGCAT-GAAGCCCACAGG	25.0 kb
199	01897-L25390	Exon 62	9430-9431	GGTCCCTGGGAG-AGAGCCATCTCG	62.6 kb
241	01364-L25393	Exon 63	9489-9490	TCAAACAACTTG-CTGGGACCATCC	37.9 kb
268	01368-L25398	Exon 64	9583-9582 reverse	TTCTGCAGTCTT-CGGAGTTTCATG	13.4 kb
312	19002-L26038	Exon 65	9781-9782	GATATGTGTCTG-AACTGGCTGCTG	3.0 kb
341	01376-L25403	Exon 66	9842-9843	TCCTGTCTTTTA-AAACTGGCATCA	2.6 kb
381	01960-L25407	Exon 67	10023-10022 reverse	GGACACTTGGCT-CAATGTTACTGC	21.1 kb
413	02482-L28035	Exon 68	10100-10101	TAGACTGGATGA-GACTGGAACCCC	2.4 kb
454	19341-L25594	Exon 69	23nt before exon 69	GAAATACATACG-TGTTTGTTTTTG	1.7 kb
493	01392-L27986	Exon 70	10421-10422	ATCCCCGAATGG-GCTACCTGCCAG	0.8 kb
166 Ж	18996-SP0733-L25739	Exon 71	38nt and 8nt before exon 71	CGGCTGAGTTTG-30nt spanning oligo-TTTTGCAGTCCC	4.4 kb
199	01949-L25418	Exon 72	10529-10530	CCCCTCAGCTTT-CACACGATGATA	1.2 kb
239	01893-L25419	Exon 73	10607-10606 reverse	GCTATCATTTAG-ATAAGATCCATT	2.9 kb
269	01902-L25423	Exon 74	10729-10730	GCCCAGATCTTG-ATTTCCTTAGAG	22.1 kb
310	01413-L25425	Exon 75	10921-10922	GCTGAGCTCATT-GCTGAGGCCAAG	1.0 kb
342	01417-L25428	Exon 76	11099-11100	CTACCTCTCTAC-AGAGGTCCGACA	12.2 kb
381	01421-L25432	Exon 77	11200-11201	CCCCAGGACACA-AGCACAGGGTTA	7.5 kb
413	19342-L25595	Exon 78	43nt after exon 78	TCTGTCTGTCTC-ATCCTGCTTTTT	6.2 kb
453	19142-L25437	Exon 79	12825-12826	CCATCCTTTGCA-TTTCTCTGCGAG	
		stop codon	*11300-11302 (Exon 79)*		

(a) The exon numbering used in this P034-B2 DMD-1 and P035-B1 DMD-2 product description is the exon numbering from the RefSeq transcript NM_004006.2, which is identical to the LRG_199 sequence. The exon numbering and NM sequence used are from 10/2018, but can be changed (by NCBI) after the release of the product description.

(b) Ligation sites of the P034 DMD-1 and P035 DMD-2 MLPA probes are indicated according to RefSeq sequence NM_004006.2 containing 79 exons.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.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.
Δ More variable. This probe is sensitive to experimental conditions. Aberrant results should be treated with caution.
± SNP rs398124074 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Related SALSA MLPA probemixes

- P176 CAPN3 Limb girdle muscular dystrophies (LGMD), contains probes for the *CAPN3* gene.
- P268 DYSF Limb girdle muscular dystrophies (LGMD), contains probes for the *DYSF* gene.
- P116 SGC Limb girdle muscular dystrophies (LGMD), contains probes for the *SGCA*, *SGCB*, *SGCD*, *SGCG* and *FKRP* genes.
- P048 LMNA Laminopathies, contains probes for the *LMNA*, *MYOT* and *CAV3* genes.

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- Gatta V et al. (2005). Identification of deletions and duplications of the DMD gene in affected males and carrier females by multiple ligation probe amplification (MLPA). *Hum Genet.* 117:92-8.
- Lee SH et al. (2015). Clinical and Genetic Characterization of Female Dystrophinopathy. *J Clin Neurol.* 11:248-51.
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P034 Product history	
Version	*Modification*
B2	Two probes have a small change in length; no change in sequence detected.
B1	All reference probes and the probes for *DMD* exons 8, 22, 23, 30, 49, 50, 65 and 69 have been replaced.
A3	The 88 and 96 nt control fragments have been replaced (QDX2).
A2	Four control fragments at 88-96-100-105 nt have been added.
A1	First release.

P035 Product history	
Version	*Modification*
B1	All reference probes and the probes for *DMD* exons 12-15, 19, 32, 33, 39, 40, 56, 71, 78, and 79 have been replaced.
A3	The 88 and 96 nt control fragments have been replaced (QDX2).
A2	Four control fragments at 88-96-100-105 nt have been added.
A1	First release.

Implemented changes in the product description
Version B2/B1-03 – 24 October 2018 (04) - Product description restructured and adapted to a new template. - Warning added to Table 1a and Table 2 for more variable probe, 262 nt probe 01958-L25397. - Warning added to Table 1b and Table 2 for SNP that could influence probe signal, 396 nt probe 01423-L01070. - Warning removed from Table 1b and Table 2, for variable probe, 239 nt probe 01893-L25419. - Several references were removed, 7 references were added. - Section on Artificial positive control DNA sample was replaced by section on Positive control DNA samples. - Minor textual changes. *Version B2/B1-02 – 10 February 2017 (03)* - Updated intended use and required specimens. - Information about ATG translation start site of Dp427c transcript added. - Information about reference sample handling added. - Information about interpretation of reference probe results added. - Website updated on page 5. - Minor textual changes. *Version B2/B1-01 – 11 May 2016 (03)* - Warning on probe 01893-L25419 modified. - Minor textual changes. *Version 31 – 05 February 2016 (02)* - Warning on probe 01893-L25419 added. *Version 30 – 26 May 2015 (02)* - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). *Version 29 – 20 December 2013 (01)* - Product description completely rewritten. - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). *Version 28 – 26 November 2013 (52)* - Warning added about mismatch near ligation site of 266 nt probe 01368-L01016.

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IVD	EUROPE* CE COLOMBIA MOROCCO ISRAEL
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and
members of the European Free Trade Association (EFTA).
The product is for RUO in all other European countries.