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

To be used with the MLPA General Protocol.

P034 version B2
For complete product history see page 12.
P035 version B1
For complete product history see page 12.

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 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.

Intended purpose
The SALSA MLPA Probemixes P034 DMD-1 and P035 DMD-2 are in vitro diagnostic (IVD)[1] or a research use only (RUO) semi-quantitative assays[2] for the detection of deletions or duplications in the *DMD* gene in genomic DNA isolated from human peripheral whole blood specimens, (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. P034 DMD-1 and P035 DMD-2 are intended to confirm a potential cause for and clinical diagnosis of Duchenne muscular dystrophy (DMD) or Becker muscular dystrophy (BMD), for molecular genetic testing of at-risk family members, and for carrier screening.

Copy number variations (CNVs) detected with P034 DMD-1 and P035 DMD-2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *DMD* gene are CNVs, however point mutations can occur which will not be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

These devices are not intended to be used for standalone diagnostic purposes, pre-implantation, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[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).
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Germline defects in the dystrophin *(DMD)* gene are the most frequent cause of Duchenne muscular dystrophy and Becker muscular dystrophy. 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. Approximately 60-70% of mutations found in patients with DMD and BMD are deletions. Duplications in the DMD gene are found in 5-15% of DMD patients and 20% of BMD patients, respectively (Duan et al. 2021). Best practice guidelines on molecular diagnostics in DMD and BMD have been published (Abbs et al. 2010, Fratter et al. 2020).

Although DMD and BMD primarily affect males due to its X-linked recessive inheritance, female carriers can occasionally manifest symptoms due to skewed X-inactivation (Korotkova et al. 2025; Sun et al. 2024; Yoshioka et al. 1998). In these cases, the X chromosome carrying the normal *DMD* gene is preferentially inactivated in a significant proportion of cells, leading to reduced dystrophin expression. This can result in a clinical phenotype ranging from mild muscle weakness to a presentation similar to DMD or BMD.

Gene structure
The *DMD* gene spans ~2.1 megabases (Mb) (NM_004006.3) on chromosome Xp21.1-p21.2 and contains 79 exons. The *DMD* LRG_199 is available at www.lrg-sequence.org and is identical to GenBank NG_012232.1.

Transcript variants
For *DMD,* multiple variants have been described. Transcript variant Dp427m encodes the main dystrophin protein found in muscle (NM_004006.3; 13992 nt; coding sequence 238-11295). *DMD* transcript variant Dp427c encodes the main dystrophin protein found in brain (NM_000109.4; 13854 nt; coding sequence 124-11157). 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.

Exon numbering
The *DMD* exon numbering used in this P034-B2 DMD-1 and P035-B1 DMD-2 product description is the exon numbering from the LRG_199 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 P034-B2 DMD-1 contains 49 MLPA probes with amplification products between 130 and 500 nucleotides (nt). The SALSA MLPA Probemix P035-B1 DMD-2 contains 48 MLPA probes with amplification products between 130 and 500 nt. The P034-B2 and P035-B1 probemixes together contain one probe for each of the 79 *DMD* exons included in transcript variant Dp427m. In addition, one probe is present in P035-B1 for the alternative promoter/exon 1 found in transcript variant Dp427c. Performing two MLPA reactions, one with P034-B2 and one with P035-B1, is thus sufficient to investigate the copy number of all *DMD* exons. In addition, P034-B2 and P035-B1 contain nine and eight reference probes, respectively, which detect

locations on the X-chromosome. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.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.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 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 peripheral whole blood specimens, (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
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 DMD or BMD. As all probes in P034 and P035 target the X chromosome the gender of the reference samples used in an experiment is not important. Target probes are first normalised to reference probes within a sample. Therefore, a ratio of 1 corresponds to 1 copy in male samples as they have one X chromosome. 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. NA05117, NA05123, NA23087, NA23094 and NA10283 from the Coriell Institute have been tested P034-B2 and P035-B1 probemixes at MRC Holland and can be used as positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Sample ID Coriell	*DMD* CNVs detected by	CNVs found
NA05117 (female)	P034	Heterozygous exon 45 deletion
NA23087 (female)	P034/P035	Heterozygous exon 2-30 duplication
NA23094 (female)	P034/P035	Heterozygous exon 35-43 deletion
NA05123 (male)	P034/P035	Exon 45-62 duplication
NA10283 (male)	P035	Exon 72-79 deletion

Performance characteristics

In most populations, approximately 80% of the mutations found in DMD are deletions/duplications which are detectable with these MLPA probemixes (Duan et al. 2021). The analytical sensitivity and specificity for the detection of deletions/duplications in Duchenne and Becker muscular dystrophy patients (based on a 2010-2021 literature review) is very high and can be considered >99%.

Analytical performance can be compromised by: SNVs or other polymorphisms 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 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.

Note: Slope correction in samples with large deletions

The slope correction algorithm in Coffalyser.Net has been optimised to give the best possible results in as many situations as possible. However, the slope correction algorithm may confuse a large *DMD* deletion and/or a deletion of a sequence targeted by a reference probe for sloping, which can lead to over- or under-correction. Since large deletions are often detected when using the P034/P035 probemixes, this issue may occur relatively frequently. Incorrectly applied slope correction can cause an FSLP warning in Coffalyser.Net or ambiguous results for multiple probes. If you suspect that slope correction was incorrectly applied, we recommend contacting info@mrcholland.com for assistance.

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 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:

Copy Number status: Male samples	Final ratio
Normal	0.80 < FR < 1.20
Deletion	FR = 0
Duplication	1.65 < FR < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	Final ratio
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 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.

P034/P035 specific notes:

- 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 deletion of one or more *DMD* exons 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 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 rearrangements 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 http://www.dmd.nl/ website has a tool to predict the effect of the deletions/duplications of exons on the reading frame. We recommend confirmation on RNA level, as the data derived from the DNA analysis does not provide absolute certainty on the reading frame effect.

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 after CNVs.
- 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. 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 kilobases (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 SNVs and unusual results (e.g., a duplication of *DMD* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1a. SALSA MLPA Probemixes 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 Δ	**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	

Table 1b. SALSA MLPA Probemixes 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 Δ	**DMD probe** 19009-L24813		**Exon 40**
490	**DMD probe** 01433-L25440		**Exon 1 of Dp427c**
500	Reference probe 10764-L25900	Xq23	

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

Δ More variable. This probe is sensitive to experimental conditions. Aberrant results should be treated with caution.

± SNP rs398124074 could influence the 396 nt probe signal (P035). SNP rs756370588 could influence the 391 nt probe signal and SNP rs398124041 could influence the 398 nt probe signal (P034). In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.
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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. DMD probes arranged according to chromosomal location

Length (nt) P034 / P035	SALSA MLPA probe	*DMD* exon[a]	Ligation site NM_004006.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon 238-240 (Exon 1)			
490	01433-L25440	Exon 1 of Dp427c	NM_000109.4; 12-13	GGCAGTAATAGA-ATGCTTTCAGGA	127.9 kb
142 Δ	01353-L25384	Exon 1 of Dp427m	55-56	TTCCCCCTACAG-GACTCAGATCTG	191.3 kb
172	01357-L25388	Exon 2	295-296	TTCAAAAGAAAA-CATTCACAAAAT	170.4 kb
213	01361-L25391	Exon 3	364-365	ACCTCTTCAGTG-ACCTACAGGATG	5.0 kb
247	01365-L25394	Exon 4	464-465	TGCCCTGAACAA-TGTCAACAAGGC	21.5 kb
284	01954-L25724	Exon 5	546-547	GTAGATGGAAAT-CATAAACTGACT	6.7 kb
319	01373-L25725	Exon 6	647-648	AACCAACAGTGA-AAAGATTCTCCT	7.0 kb
357	01713-L25404	Exon 7	794-795	CTGGAATAGTGT-GGTTTGCCAGCA	110.5 kb
391 ±	19004-L24808	Exon 8	10 nt after exon 8	AGGTAAAGTGTG-TAAAGGACAGCT	1.1 kb
427	01385-L25412	Exon 9	1095-1096	GCACAGGGATAT-GAGAGAACTTCT	52.8 kb
469	01718-L26101	Exon 10	1224-1225	GAAGACAAGTCA-TTTGGCAGTTCA	0.8 kb
141	01393-L25414	Exon 11	1411-1412	ATTTGACAGCCC-ATCAGGGCCGGG	29.9 kb
171 Ж	18997-SP0734-L25740	Exon 12	1603-1604; 1639-1640	TCCAGAATCAGA-36 nt spanning oligo-AAGAAAGAACAA	18.6 kb
211	19001-L24805	Exon 13	1839-1838 reverse	AATAATCTGACC-TTAAGTTGTTCT	21.9 kb
247	19338-L25591	Exon 14	7 nt before exon 14	TTGATTGTCTCT-TCTCCAGGTATT	0.3 kb
283	19141-L25730	Exon 15	2003-2004	TCACACAACTGG-CTTTAAAGATCA	7.7 kb
319	02060-L25426	Exon 16	2088-2089	AAGCAATCCATG-GGCAAACTGTAT	20.6 kb
358	01952-L25429	Exon 17	2321-2322	GGAACAGATCCT-GGTAAAGCATGC	27.2 kb
388	01891-L25433	Exon 18	2462-2463	AGAAGCTGTGTT-GCAGAGTCCTGA	16.2 kb
427	19005-L24809	Exon 19	9 nt before exon 19	ATCTTTGCTCTC-ATGCTGCAGGCC	10.4 kb
466	01430-L25438	Exon 20	2679-2680	CGGTGGATCGAA-TTCTGCCAGTTG	6.4 kb
157	01355-L25386	Exon 21	2945-2946	GAAAGGACAAGG-ACCCATGTTCCT	12.8 kb
193	18998-L24802	Exon 22	3159-3160	GACTATGAAATC-ATGGAGCAGAGA	3.7 kb
226	19138-L25106	Exon 23	3393-3394	AAACTCCGAAAA-ATTCAGGTAATT	3.9 kb
262 Δ	01958-L25397	Exon 24	3470-3471	ATGGCCTGCCCT-TGGGGATTCAGA	1.1 kb
303	01371-L25399	Exon 25	3583-3584	GGCAGAAGATAA-AGAATGAAGCAG	8.8 kb
332	01375-L25402	Exon 26	3724-3725	CTGTAAGCCTCC-AGAAAGATCTAT	6.2 kb
373	01379-L25406	Exon 27	3900-3901	ACTGAGTCTGTA-AATAGTGTCATA	7.3 kb
405	01716-L25410	Exon 28	4049-4050	TTGGCATGAGTT-ATTGTCATACTT	3.0 kb
445	01387-L25413	Exon 29	4227-4228	GCACAGACCCTA-ACAGATGGCGGA	26.5 kb
483	19008-L24812	Exon 30	4430-4431	AGCTTATATTGC-AGACAAGGTGGA	21.7 kb
157	01395-L25416	Exon 31	4548-4549	AAGGAGGCTGCC-CAAAGAGTCCTG	0.6 kb
193	18999-L24803	Exon 32	4735-4736	AAGTAGTACAGT-CACAGCTAAATC	3.2 kb

Length (nt) P034 / P035	SALSA MLPA probe	*DMD* exon[a]	Ligation site NM_004006.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
226	19140-L25108	Exon 33	4882-4883	TAACAGCTTTGA-AATTGCATTATA	5.7 kb
263	01901-L25422	Exon 34	4976-4977	GCGAAAGGAAAT-GAATGTCTTGAC	15.5 kb
303	15720-L25424	Exon 35	5127-5128	CACCTGAAGAGT-ATCACAGAGGTA	0.5 kb
332	01416-L25427	Exon 36	5317-5318	ACATCACAAAGT-GGATCATTCAGG	1.8 kb
372	03038-L26168	Exon 37	5527-5526 reverse	AATGGCTGCAAA-TCGATGGTTGAG	14.4 kb
407	19372-L25772	Exon 38	5642-5643	GGCTGAAATTCA-GCAGGGGGTGAA	2.4 kb
445	19007-L24811	Exon 39	4 nt before exon 39	TGTTGTTTTTGA-TCAGAATGAAGA	2.8 kb
481 Δ	19009-L24813	Exon 40	23 nt before exon 40	TATTGATATTTT-AATAATGTCTGC	1.1 kb
149	18831-L25385	Exon 41	6048-6049	GAGGGCTTGTCT-GAGGATGGGGCC	32.0 kb
187	01711-L25389	Exon 42	6188-6189	AACGATGATGGT-GATGACTGAAGA	22.6 kb
220	18221-L25392	Exon 43	6432-6433	GCATTGCAAAGT-GCAACGCCTGTG	70.6 kb
254	01366-L25396	Exon 44	6618-6619	GAACAGTTTCTC-AGAAAGACACAA	248.6 kb
291	01370-L01287	Exon 45	6774-6775	ACAGATGCCAGT-ATTCTACAGGAA	36.3 kb
325	01374-L25401	Exon 46	6942-6943	AACATTGCTAGT-ATCCCACTTGAA	2.4 kb
364	01378-L25405	Exon 47	7046-7047	TCTCAAACAATT-AAATGAAACTGG	54.4 kb
398 ±	01382-L25408	Exon 48	7254-7255	CAGTTAAATCAT-CTGCTGCTGTGG	38.4 kb
436	19006-L24810	Exon 49	10 nt before exon 49	CTATATGGGTTC-TTTTCCCCAGGA	16.9 kb
476	19340-L25774	Exon 50	13 nt after exon 50 reverse	AGAGAATGGGAT-CCAGTATACTTA	45.9 kb
148	01394-L25415	Exon 51	7659-7660	GCTCTGGCAGAT-TTCAACCGGGCT	44.4 kb
187	02059-L25417	Exon 52	7866-7865 reverse	CTAGCCTCTTGA-TTGCTGGTCTTG	50.2 kb
219	01892-L01050	Exon 53	8023-8024	CTGAGCAGGTCT-TAGGACAGGCCA	21.3 kb
254	01894-L25421	Exon 54	8145-8146	TGGCAGACAAAT-GTAGATGTGGCA	30.3 kb
291	01411-L01058	Exon 55	8305-8306	AAACTCATAGAT-TACTGCAACAGT	120.6 kb
325 Ж	19003-SP0735-L24807	Exon 56	8620-8621; 27 nt after exon 56	AAAAGTCTCTCA-34 nt spanning oligo-CACAAATGAATT	10.4 kb
364	01419-L25430	Exon 57	8715-8716	CTGAAAGATGAT-GAATTAAGCCGG	17.8 kb
396 ±	01423-L01070	Exon 58	8846-8847	TGAGACTGTACG-AATATTTCTGAC	0.8 kb
436	01427-L25436	Exon 59	9034-9035	TAGATGAGACCC-TTGAAAGACTCC	33.6 kb
472	01431-L25439	Exon 60	9204-9205	GCGCCTCTGAAA-GAGAACGTGAGC	96.0 kb
165	01356-L25387	Exon 61	9354-9355	AGGCAGCTGCAT-GAAGCCCACAGG	25.0 kb
199	01897-L25390	Exon 62	9423-9424	GGTCCCTGGGAG-AGAGCCATCTCG	62.6 kb
241	01364-L25393	Exon 63	9482-9483	TCAAACAACTTG-CTGGGACCATCC	37.9 kb
268	01368-L25398	Exon 64	9576-9575 reverse	TTCTGCAGTCTT-CGGAGTTTCATG	13.6 kb
312	19002-L26038	Exon 65	9774-9775	GATATGTGTCTG-AACTGGCTGCTG	2.9 kb
341	01376-L25403	Exon 66	9835-9836	TCCTGTCTTTTA-AAACTGGCATCA	2.6 kb
381	01960-L25407	Exon 67	10016-10015 reverse	GGACACTTGGCT-CAATGTTACTGC	21.1 kb
413	02482-L28035	Exon 68	10093-10094	TAGACTGGATGA-GACTGGAACCCC	2.4 kb
454	19341-L25594	Exon 69	23 nt before exon 69	GAAATACATACG-TGTTTGTTTTTG	1.8 kb
493	01392-L27986	Exon 70	10414-10415	ATCCCCGAATGG-GCTACCTGCCAG	0.7 kb
166 Ж	18996-SP0733-L25739	Exon 71	38 nt before exon 71; 8 nt before exon 71	CGGCTGAGTTTG-30 nt spanning oligo-TTTTGCAGTCCC	4.4 kb
199	01949-L25418	Exon 72	10522-10523	CCCCTCAGCTTT-CACACGATGATA	1.2 kb
239	01893-L25419	Exon 73	10600-10599 reverse	GCTATCATTTAG-ATAAGATCCATT	2.9 kb
269	01902-L25423	Exon 74	10722-10723	GCCCAGATCTTG-ATTTCCTTAGAG	22.1 kb

Length (nt) P034 / P035	SALSA MLPA probe	*DMD* exon[a]	Ligation site NM_004006.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
310	01413-L25425	Exon 75	10914-10915	GCTGAGCTCATT-GCTGAGGCCAAG	1.0 kb
342	01417-L25428	Exon 76	11092-11093	CTACCTCTCTAC-AGAGGTCCGACA	12.2 kb
381	01421-L25432	Exon 77	11193-11194	CCCCAGGACACA-AGCACAGGGTTA	7.6 kb
413	19342-L25595	Exon 78	43 nt after exon 78	TCTGTCTGTCTC-ATCCTGCTTTTT	6.2 kb
453	19142-L25437	Exon 79	12818-12819	CCATCCTTTGCA-TTTCTCTGCGAG	
	stop codon 11293-11295 (Exon 79)				

[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.

Δ More variable. This probe is sensitive to experimental conditions. Aberrant results should be treated with caution.
± SNP rs398124074 could influence the 396 nt probe signal (P035). SNP rs756370588 could influence the 391 nt probe signal and SNP rs398124041 could influence the 398 nt probe signal (P034). In case of apparent deletions, it is recommended to sequence the regions targeted by these probes.
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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA Probemixes

For related products, see the product pages of P034 DMD-1 and P035 DMD-2 on our website.

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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/B1-09 – 03 June 2025 (04P)
- Added the abbreviations for Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) to the intended purpose.
- Added information on female carriers that can manifest symptoms due to skewed X-inactivation to the clinical background section.
- Related SALSA MLPA products section replaced with reference to product page on website.
- Added three publications to the References section.

Version B2/B1-08 – 19 January 2023 (04P)
- Section Note: Slope correction in samples with large deletions updated.
- Section of P034/P035 specific notes updated.

- Warning added to Table 1 and Table 2 for more variable probes, 142 nt probe 01353-L25384, and 481 nt probe 19009-L24813.
- Various minor textual changes.

Version B2/B1-07 – 16 September 2021 (04P)
- Section of P034/P035 specific notes updated.

Version B2/B1-06 – 24 March 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended purpose updated.
- Clinical background and Performance characteristics sections updated.
- Reference samples section updated.
- Note for slope correction added to the data analysis section.
- Section of selected publications updated.
- UK added to the list of countries in Europe that accept the CE mark.

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RUO	ALL OTHER COUNTRIES

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