Product Description
SALSA® MLPA® Probemix P326-B1 LARGE1

To be used with the MLPA General Protocol.

Version B1
As compared to version A2, two target probes have been replaced, three target probes have been added, four reference probes have been replaced and 28 probes have been changed in length, not in the sequence detected. For complete product history see page 8.

Catalogue numbers:
- **P326-025R:** SALSA MLPA Probemix P326 LARGE1, 25 reactions.
- **P326-050R:** SALSA MLPA Probemix P326 LARGE1, 50 reactions.
- **P326-100R:** SALSA MLPA Probemix P326 LARGE1, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information
The SALSA MLPA Probemix P326 LARGE1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *LARGE1*, *FKTN* and *POMT2* genes. Defects in these genes are associated with forms of congenital or limb-girdle muscular dystrophy in which obstruction of O-linked glycosylation is cause of disease, collectively referred to as dystroglycanopathies (Martin 2005). Dystroglycanopathies are autosomal recessive disorders and include Walker-Warburg syndrome, muscle-eye-brain disease, Fukuyama congenital muscular dystrophy, congenital muscular dystrophy 1C and 1D, and limb girdle muscular dystrophy 2I. Mutations in the *FKTN* gene may also result in dilated cardiomyopathy without muscular dystrophic features (Murakami et al. 2006).

Although point mutations may be a more common cause of gene disfunction, intragenic deletions have been reported for *LARGE1* (van Reeuwijk et al. 2007), *FKTN* (Hobbiebrunken et al. 2016) and *POMT2* (Yanagisawa et al. 2009).

More information on *LARGE1* is available at https://www.omim.org/entry/603590
More information on *FKTN* is available at https://www.omim.org/entry/607440
More information on *POMT2* is available at https://www.omim.org/entry/607439

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 *LARGE1* exon numbering used in this P326-B1 LARGE1 product description is the exon numbering from the NG_009929.2 sequence. The *FKTN* exon numbering used in this P326-B1 LARGE1 product description is the exon numbering from the LRG_434 sequence. The *POMT2* exon numbering used in this P326-B1 LARGE1 product description is the exon numbering from the LRG_844 sequence.

The exon numbering of the NM_ sequences that were used for determining a probe's ligation site do not always correspond to the exon numbering obtained from the NG sequences or LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequences and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P326-B1 LARGE1 contains 55 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 30 probes for the *LARGE1* gene and one probe targeting a sequence located upstream of the *LARGE1* gene, six probes for the *FKTN* gene, and nine probes for the *POMT2* gene. 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.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 is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of dystroglycanopathies. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.

- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- The major cause of genetic defects in the *LARGE1, FKTN* and *POMT2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P326 LARGE1.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

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

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

Mutation databases

***LARGE1*:** https://databases.lovd.nl/shared/genes/LARGE
FKTN: https://databases.lovd.nl/shared/genes/FKTN
***POMT2*:** https://databases.lovd.nl/shared/genes/POMT2
We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *LARGE1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P326-B1 LARGE1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*LARGE1*	*FKTN*	*POMT2*
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 03896-L00020	11q			
137 ¥	**FKTN probe** 12413-L32231			**Exon 1**	
144 *	**LARGE1 probe** 22809-L32339		**Exon 2**		
148	**POMT2 probe** 12415-L13416				**Exon 1**
154	**LARGE1 probe** 12416-L13417		**Exon 12**		
162 ¥ «	**LARGE1 probe** 12417-L32232		**Exon 1**		
168 ¥	**FKTN probe** 12418-L26256			**Exon 11**	
175 ¥	**POMT2 probe** 12419-L32233				**Exon 8**
178	**LARGE1 probe** 12420-L13421		**Exon 3**		
184	**FKTN probe** 12421-L13422			**Exon 4**	
193 ¥	**POMT2 probe** 12422-L28134				**Exon 3**
199 ¥	Reference probe 09629-L32234	17q			
203 ¥	**LARGE1 probe** 12424-L32340		**Exon 11**		
208 ¥	**FKTN probe** 12425-L32235			**Exon 7**	
218 ¥	**POMT2 probe** 12426-L32236				**Exon 6**
225 ¥	**LARGE1 probe** 12427-L32341		**Exon 8**		
230 ¥	Reference probe 09100-L32342	4q			
235 ¥	**LARGE1 probe** 12429-L32237		**Exon 6**		
241 ¥	**FKTN probe** 12430-L32238			**Exon 6**	
246 ¥	**LARGE1 probe** 12431-L32343		**Exon 15**		
250	**LARGE1 probe** 12432-L13433		**Exon 5**		
256 «	**LARGE1 probe** 12433-L14686		**Exon 1**		
262	**POMT2 probe** 12434-L13435				**Exon 21**
266	**LARGE1 probe** 12435-L13436		**Exon 15**		
274	**LARGE1 probe** 12436-L13437		**Exon 4**		
279	**POMT2 probe** 12437-L13438				**Exon 4**
286	**LARGE1 probe** 12438-L13439		**Exon 10**		
293 ¥	Reference probe 02414-L32344	16q			
299 ¥	**FKTN probe** 12439-L32345			**Exon 8**	
306 ¥	**LARGE1 probe** 12441-L32239		**Exon 4**		
312 ¥	**POMT2 probe** 12442-L26378				**Exon 12**
319 ¥	**LARGE1 probe** 12443-L32346		**Exon 7**		
327 ¥	**POMT2 probe** 12444-L32347				**Exon 15**
332	**LARGE1 probe** 12445-L13446		**Exon 5**		
338	Reference probe 09260-L11427	7q			
344 *	**LARGE1 probe** 22810-L32173		**Exon 7**		
350 ¥	**LARGE1 probe** 12447-L32240		**Exon 12**		
358 ¥	**LARGE1 probe** 12448-L32241		**Exon 16**		
364	**POMT2 probe** 12449-L13450				**Exon 18**
373 ¥	**LARGE1 probe** 12450-L28963		**Exon 13**		
385 ¥	**LARGE1 probe** 12451-L32242		**Exon 2**		
393 *	Reference probe 04838-L04222	5p			
402 ¥	**LARGE1 probe** 12453-L32243		**Exon 14**		
409 ¬	**EP300 probe** 12280-L13223		**Upstream**		
418 *	Reference probe 09793-L25209	15q			
427	**LARGE1 probe** 12456-L13457		**Exon 11**		
436	**LARGE1 probe** 12457-L13458		**Exon 10**		
442 *	**LARGE1 probe** 22811-L32348		**Exon 6**		
452 ¥	**LARGE1 probe** 12458-L32244		**Exon 16**		
457 *	Reference probe 17634-L32373	21q			
465 ¥	**LARGE1 probe** 12460-L32162		**Exon 9**		

480 ¥	**LARGE1 probe** 12461-L32245		**Exon 3**		
485 *	**LARGE1 probe** 22812-L32175		**Exon 8**		
493 *	**LARGE1 probe** 22813-L32176		**Exon 13**		
500 *	Reference probe 09682-L22509	3p			

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

* New in version B1.
¥ Changed in version B1. Minor alteration, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P326-B1 probes arranged according to chromosomal location

Table 2a. *LARGE1*

Length (nt)	SALSA MLPA probe	*LARGE1* exon[a]	Ligation site NM_004737.7	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
409 ¬	12280-L13223	*EP300* gene		ACCACCAACAAC-ACAACTTCCCCA	7.2 **M**b
		start codon	*628-630 (Exon 3)*		
256 «	12433-L14686	Exon 1	117-116 *reverse*	CCGCGCTCCAAG-CCGATTCGTCCC	0.6 kb
162 «	12417-L32232	Exon 1	245 nt after exon 1 reverse	TCCTCCTGGCTC-GCTCCCTGCCAG	63.0 kb
144	22809-L32339	Exon 2	13 nt before exon 2	TAAAGGGGCCTT-GTCTATTTTTCA	0.1 kb
385	12451-L32242	Exon 2	71 nt after exon 2	TTGCCGTTTTTC-AGCAGGGCGATC	95.1 kb
480	12461-L32245	Exon 3	36 nt before exon 3	ATCTCCAGTGCT-AAGAACTCAGGC	0.2 kb
178	12420-L13421	Exon 3	708-709	ATCACCTGGATT-TACCTGTTTTCT	110.6 kb
274	12436-L13437	Exon 4	82 nt before exon 4	GCCTCGCCATGT-AGTAAGGGATGT	0.4 kb
306	12441-L32239	Exon 4	1028-1029	GGTCGTGGAGAA-ATGCGAGGTAAA	23.8 kb
332	12445-L13446	Exon 5	257 nt before exon 5	TATTTGAGCCAA-TCACCATCTCTC	0.3 kb
250	12432-L13433	Exon 5	2 nt after exon 5	TGTTCCATAGGT-AAGAACAACTTC	21.7 kb
442	22811-L32348	Exon 6	1142-1143	GCACTTCCACCT-TATTGCTGACTC	0.1 kb
235	12429-L32237	Exon 6	1233-1234	TACAATGCAGAC-GAGCTCAAGGTA	39.5 kb
344	22810-L32173	Exon 7	1323-1324	AAGACTCTTCCT-GCCAACCTGGAG	0.1 kb
319	12443-L32346	Exon 7	17 nt after exon 7 reverse	CGGGGAAAAAAC-GAATGGGCTACC	132.6 kb
485	22812-L32175	Exon 8	3 nt before exon 8	CTCTGTACCTCA-CAGGTCAGCAAG	0.1 kb
225	12427-L32341	Exon 8	9 nt after exon 8	CAGGTAAATCCT-CAGGGTGGTATG	48.0 kb
465	12460-L32162	Exon 9	22 nt after exon 9 reverse	AGGAATAGCTGC-ACCTTCGAACCT	2.2 kb
436	12457-L13458	Exon 10	1691-1692	GCTCCCCTGCTT-CTGGAATGTGCA	0.1 kb
286	12438-L13439	Exon 10	1755-1756	GTGTCTGATCTA-AAGGTAGGGTCA	44.1 kb
427	12456-L13457	Exon 11	1762-1763	CCTGCCAGGTCA-TTCACTGGAACT	0.1 kb
203	12424-L32340	Exon 11	1898-1899	CAGTGAGGCTGA-TGTCAACAGTGA	21.4 kb
154	12416-L13417	Exon 12	1933-1934	AGCAGCTGTCTG-AGCTGGACGAGG	0.1 kb
350	12447-L32240	Exon 12	2009-2010	CCTGTACTTCCT-GCACTACGAGTA	11.8 kb
493	22813-L32176	Exon 13	2235-2236	ATCGTGTACAAG-GAGGGCCAGTTC	0.1 kb
373	12450-L28963	Exon 13	2 nt after exon 13	AGTACCTCAGGT-AAGGACCTGCAG	20.9 kb
402	12453-L32243	Exon 14	2376-2377	GTCATCCAGCTC-GATCTTGCCAAC	6.1 kb
266	12435-L13436	Exon 15	2521-2520 reverse	TGCGTGGCCTTT-CGTCCAGACGTG	0.2 kb
246	12431-L32343	Exon 15	28 nt after exon 15	ATCCACCTGGTA-TGGTCGACGGGG	2.5 kb
358	12448-L32241	Exon 16	2816-2817	TCTCAAAACCCT-CAAGGAAGAGTT	1.1 kb

452	12458-L32244	Exon 16	3959-3960	CAGGAAGAAAGT-GATTGGGTTCCT	
		stop codon	*2896-2898 (Exon 16)*		

Table 2b. *FKTN*

Length (nt)	SALSA MLPA probe	*FKTN* exon[a]	Ligation site NM_001079802.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*216-218 (Exon 3)*		
137	12413-L32231	Exon 1	56 nt after exon 1	GCCTTCAGACAG-GTACCGCTGGCG	38.4 kb
184	12421-L13422	Exon 4	358-359	AGGAAGCCGAAT-TGGATTTGATAG	7.9 kb
241	12430-L32238	Exon 6	862-863	AGCTTTTGACAG-GTAAGTTCAGAG	3.3 kb
208	12425-L32235	Exon 7	879-880	AGTTACAGCAAG-TTACTGTTGATG	7.5 kb
299	12439-L32345	Exon 8	1052-1053	AGTGCAAAGGAA-TTACTGCAACTA	23.1 kb
168	12418-L26256	Exon 11	4779-4780	TCACATCTGGAG-ACATCAGCCTTT	
		stop codon	*1599-1601 (Exon 11)*		

Table 2c. *POMT2*

Length (nt)	SALSA MLPA probe	*POMT2* exon[a]	Ligation site NM_013382.7	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*203-205 (Exon 1)*		
148	12415-L13416	Exon 1	259 nt after exon 1	GCTACAGTGCAT-CACCTGGCTTGC	13.8 kb
193	12422-L28134	Exon 3	558-559	TCTTGCTGGCTA-CCTGAGTGGATA	1.7 kb
279	12437-L13438	Exon 4	689-690	CCTACCTCACTG-TACTGGATCTGT	3.2 kb
218	12426-L32236	Exon 6	288 nt before exon 6	CTGGTCACTTGT-GAAGAAGGAGCC	2.8 kb
175	12419-L32233	Exon 8	1196-1197	TGCACAATGCTT-CCATCCCTGAAC	12.1 kb
312	12442-L26378	Exon 12	173 nt after exon 12	TCTGGGAAAAGC-AGCCTATGAAGT	2.8 kb
327	12444-L32347	Exon 15	1845-1846	ATCCCACATGGT-CATGATCCGGGT	4.1 kb
364	12449-L13450	Exon 18	136 nt after exon 18 reverse	GGTTGTTACCTT-TCAAAAGCAAAG	4.6 kb
262	12434-L13435	Exon 21	4723-4724	CACTTGGCAGAA-ACGTGATGTGTC	
		stop codon	*2453-2455 (Exon 21)*		

[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.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P048 LMNA/MYOT/ZMPSTE24	Contains probes for the *LMNA*, *MYOT*, *ZMPSTE24* and *CAV3* genes, involved in laminopathies.
P116 SGC	Contains probes for the *SGCA*, *SGCB*, *SGCD*, *SGCG* and *FKRP* genes involved in LGMD2D, 2E, 2F, 2C, and 2I.
P176 CAPN3	Contains probes for the *CAPN3* gene, involved in LGMD2A.
P268 DYSF	Contains probes for the *DYSF* gene, involved in LGMD2B.

References

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- Martin PT. (2005) The dystroglycanopathies: the new disorders of O-linked glycosylation. *Semin Pediatr Neurol.* 12:152-158.
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- van Reeuwijk J et al. (2007). Intragenic deletion in the *LARGE* gene causes Walker-Warburg syndrome. *Hum Genet.* 121:685-690.
- 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.
- Yanagisawa A et al. (2009). *POMT2* intragenic deletions and splicing abnormalities causing congenital muscular dystrophy with mental retardation. *Eur J Med Genet*. 52:201-206.

Selected publications using SALSA MLPA Probemix P326 LARGE1

- Costa C et al. (2013). A Portugese case of Fukuyama congenital muscular dystrophy caused by a multiexonic duplication in the fukutin gene. *Neuromuscul Disord*. 23:557-561.
- Oliveira J et al. (2015). New splicing mutation in the choline kinase beta (CHKB) gene causing a muscular dystrophy detected by whole-exome sequencing. *J Hum Genet.* 60:305-312.

P326 product history	
Version	*Modification*
B1	Two target probes have been replaced, three target probes have been added, four reference probes have been replaced and 28 probes have been changed in length, not in the sequence detected.
A2	One reference probe has been replaced and the control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description
Version B1-01 – 12 January 2021 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *LARGE1*, *FKTN* and *POMT2* genes updated according to new version of the NM_ reference sequence. Version 08 – 14 June 2017 (55) - Product description adapted to a new product lot (lot number changed, lot number added, small changes in Table 1 and 2, new picture included). - Change gene name LARGE into LARGE1 (including change name of probemix) - NM sequence LARGE1 updated, which results in update of position of ligation sites in Table 2.

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