SALSA MLPA probemix P176-C3 CAPN3

Lot C3-0517: As compared to version C2-0813, three reference probes have been replaced and three probe lengths have been adjusted.

Limb-girdle muscular dystrophies (LGMD) are a group of phenotypically and genotypically heterogeneous diseases, characterised by progressive weakness and atrophy of the muscles of the pelvic and shoulder girdle. Mutations of the CAPN3 gene have been associated with limb-girdle muscular dystrophy type 2A (LGMD2A). Patients with LGMD2A have symmetrical and selective involvement of proximal limb-girdle muscles. The disease shows wide intrafamilial and interfamilial clinical variability. The age at onset ranges from 2 to 40 years, but the disease usually first appears in the second or third decade of life, with the development of proximal weakness in the lower limbs. Mutations in CAPN3 result in a cascade of events leading eventually to muscular dystrophy, but the precise underlying mechanisms have yet to be elucidated. However, a defect of calpain 3, the protein encoded by CAPN3, proteolytic activity is largely recognised as the main pathogenic cause of LGMD2A.

The CAPN3 gene (24 exons) spans ~53 kb of genomic DNA and is located on chromosome 15q15.1, ~40 Mb from the p-telomere. The gene is predominantly expressed in skeletal muscle where it is present in the cytosol as well as in the nucleus. The protein encoded by this gene (calpain 3) belongs to the superfamily of calcium-activated neutral proteases, which are non-lysosomal intracellular cysteine proteases. Calpains respond to Ca^{2+} signals by cleaving specific proteins, frequently components of signalling cascades, thereby irreversibly modifying their function(s).

The P176-C3 probemix contains probes for each of the 24 CAPN3 exons. Two probes are present for exons 1 and 4. In addition, ten reference probes are included, detecting several different autosomal chromosomal locations.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned gene in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Finally, note that most defects in these genes are expected to be small (point) mutations, most of which will not be detected by this SALSA® MLPA® test.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of a SALSA® MLPA® probemix and reagents requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

More information

Website : www.mlpa.com
E-mail : info@mlpa.com (information & technical questions); order@mlpa.com (for orders)
Mail : MRC-Holland bv; Willem Schoutenstraat 1, 1057 DL Amsterdam, the Netherlands

Related SALSA® MLPA® probemixes

- P268 DYSF: Contains probes for the DYSF gene involved in LGMD2B.
- P116 SGC: Contains probes for the SGCA, SGCB, SGCD, SGCG and FKRP genes involved in LGMD2D, 2E, 2F, 2C, and 2I.
- P061 Lissencephaly: Contains probes for the POMT1 gene involved in LGMD2K.
- P048 LMNA/MYOT: Contains probes for the LMNA, MYOT and CAV3 genes.
- P034/P035 DMD: Duchenne muscular dystrophy and Becker muscular dystrophy. Contains probes for all DMD exons.
- P436 ANO5: Contains probes for the ANO5 gene, involved in LGMD2L

References

- Stehlíková, K et al., 2014. Autosomal recessive limb-girdle muscular dystrophies in the Czech Republic. *BMC Neurol* 14.1: 154.

Data analysis

The P176-C3 CAPN3 probemix contains 36 MLPA probes with amplification products between 130 and 427 nt. In addition, it contains 9 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix can first be normalised intra-sample by dividing the peak height of each probe's amplification product by the total peak height of only the reference probes in this probemix (block normalisation). Secondly, inter-sample normalisation can be achieved by dividing this intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes no changes occurred in the genomic regions recognised by the reference probes.

Data normalisation should be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blotting, long range PCR, qPCR, FISH.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. For example, a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

This probemix was developed at MRC-Holland.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1. SALSA MLPA P176-C3 CAPN3 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position reference	Chromosomal position CAPN3
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation		
100	X-fragment: Specific for the X chromosome		
105	Y-fragment: Specific for the Y chromosome		
130	Reference probe 00797-L00463	5q31	
142	**CAPN3 probe** 05785-L05232		**Exon 1**
148	**CAPN3 probe** 05795-L05242		**Exon 10**
154	Reference probe 03857-L03308	17q11	
160	**CAPN3 probe** 05787-L05234		**Exon 2**
166 ¥	**CAPN3 probe** 21484-L30121		**Exon 11**
178	**CAPN3 probe** 05788-L05235		**Exon 3**
184	**CAPN3 probe** 05797-L05244		**Exon 13**
190	**CAPN3 probe** 10611-L11162		**Exon 15**
196	Reference probe 03547-L02913	11p15	
203	**CAPN3 probe** 05789-L05236		**Exon 4**
211	**CAPN3 probe** 05798-L05245		**Exon 16**
221	Reference probe 01827-L01392	16p13	
229	**CAPN3 probe** 05790-L05237		**Exon 5**
238	**CAPN3 probe** 05799-L05246		**Exon 17**
247	Reference probe 02317-L01808	19p13	
256	**CAPN3 probe** 05791-L05238		**Exon 6**
265	**CAPN3 probe** 05800-L13102		**Exon 18**
275 *	Reference probe 12494-L13538	1q32	
283	**CAPN3 probe** 05792-L05239		**Exon 7**
292	**CAPN3 probe** 10613-L11164		**Exon 20**
301	**CAPN3 probe** 10615-L11166		**Exon 23**
308	**CAPN3 probe** 17899-L11157		**Exon 24**
315 *	Reference probe 06741-L24262	8q12	
320 ¥	**CAPN3 probe** 05802-L20124		**Exon 22**
330 ¥	Reference probe 01918-L21732	1q22	
337	**CAPN3 probe** 05794-L05241		**Exon 9**
347	**CAPN3 probe** 17898-L11156		**Exon 8**
355	**CAPN3 probe** 10610-L11161		**Exon 14**
364	**CAPN3 probe** 05786-L05233		**Exon 1**
382	**CAPN3 probe** 10614-L11165		**Exon 21**
391 ±	**CAPN3 probe** 10609-L11160		**Exon 12**
400 *	Reference probe 15766-L24901	14q32	
409	**CAPN3 probe** 10606-L11158		**Exon 4**
418	**CAPN3 probe** 10612-L11163		**Exon 19**
427	Reference probe 05561-L04993	7p14	

¥ Changed in version C3 (from lot C3-0517 onwards). Small change in length, no change in sequence detected.

* New in version C3 (from lot C3-0517 onwards).

± SNP (rs28364489) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Notes

- **The CAPN3 exon numbering has changed.** From description version 11 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for this gene. This exon numbering used here may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2.

The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. CAPN3 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	CAPN3 exon	Ligation site NM_000070.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*307-309 (exon 1)*		
142	05785-L05232	Exon 1	455-456	CATCAGCCGCAA-TTTTCCTATTAT	0.1 kb
364	05786-L05233	Exon 1	573-574	TCTCTCTTTTAT-AGCCAGAAGTTC	24.5 kb
160	05787-L05234	Exon 2	1 nt after exon 2	GGAGAGCTAGGT-AGGAAAGTGCCT	1.7 kb
178	05788-L05235	Exon 3	759-760	GTCATACCCCAT-GATCAAAGTTTC	1.4 kb
409	10606-L11158	Exon 4	102 nt before exon 4	TCCAGGAAATGA-TGCTGCTTTGGG	0.1 kb
203	05789-L05236	Exon 4	816-817	TTCTGGCGCTAT-GGAGAGTGGGTG	1.3 kb
229	05790-L05237	Exon 5	1035-1036	GAGATCAGGGAT-GCTCCTAGTGAC	1.0 kb
256	05791-L05238	Exon 6	1148-1149	TCCTTCTGGTCT-GAACATGGGGGA	2.7 kb
283	05792-L05239	Exon 7	1272-1273	CCGGTTCAGTAT-GAGACAAGAATG	1.6 kb
347	17898-L11156	Exon 8	25 nt before exon 8	GGCTGCAGAGCA-TGAGAGCTCTTT	2.6 kb
337	05794-L05241	Exon 9	1442-1443	CTGGAGCTTTGT-GGACAAAGATGA	2.7 kb
148	05795-L05242	Exon 10	1549-1550	TGGAGATCTGCA-ACCTCACGGCCG	2.1 kb
166	21484-L30121	Exon 11	1700-1701	TCTGAAGCTCCT-GGAGGAGGACGA	0.4 kb
391 ±	10609-L11160	Exon 12	21 nt before exon 12	TCTGAAGCATCT-TCCTTTCTGTTT	0.8 kb
184	05797-L05244	Exon 13 (14)	1917-1918	AGCAAAACCTAC-ATCAACATGCGG	0.9 kb
355	10610-L11161	Exon 14 (15)	2058-2059	CACAGGGAAGTT-GAAAATACCATC	2.3 kb
190	10611-L11162	Exon 15 (17)	84 nt after exon 15	GTGTGAGCTCAT-ATGCATCCATGC	2.2 kb
211	05798-L05245	Exon 16 (18)	2137-2138	ACAGAGCAAACA-GCAACAAGGAGC	1.1 kb
238	05799-L05246	Exon 16 (19)	2262-2263	GAGGAACAGCAA-CAATTCCGGAAC	0.5 kb
265	05800-L13102	Exon 18 (20)	2319-2320	ATCTGTGCAGAT-GAGCTCAAGAAG	0.2 kb
418	10612-L11163	Exon 19 (21)	2387-2388	CGGGTTCACACT-GGAGTCCTGCCG	0.5 kb
292	10613-L11164	Exon 20 (22)	6 nt after exon 20	GGCAGGTGGGAA-GAGAAAATGAAG	0.1 kb
382	10614-L11165	Exon 21 (23)	2551-2552	ACGAGATGCGAA-ATGCAGTCAACG	0.3 kb
320	05802-L20124	Exon 22 (24)	2595-2596	AACCAGCTCTAT-GACATCATTACC	0.4 kb
301	10615-L11166	Exon 23 (25)	2707-2708	ATGCATTTGACA-AGGATGGAGATG	0.5 kb
308	17899-L11157	Exon 24 (26)	2762-2763	GCAGCTCACCAT-GTATGCCTGAAC	
		Stop codon	*2770-2772 (exon 24)*		

± SNP (rs28364489) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

The NM_000070.2 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Note: The CAPN3 exon numbering has changed. From description version 11 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for this gene. The exon numbering used in previous versions of this product description can be found between brackets in Table 2. Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P176-C3 CAPN3 sample picture

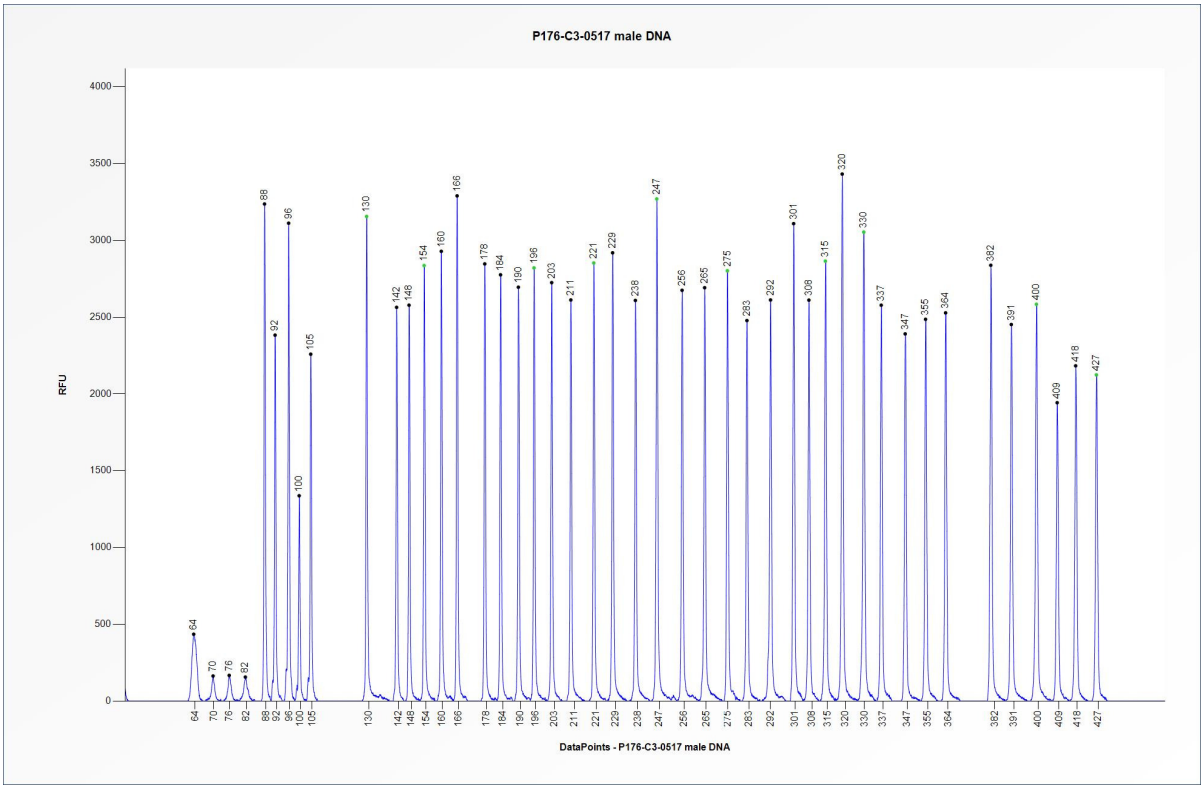

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P176-C3 CAPN3 (lot C3-0517).

Implemented Changes – compared to the previous product description versions
Version 11 – 27 June 2017 (55) - Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included). - Various minor textual changes on pages 1 and 2. - New reference added on page 2. - Exon numbering of CAPN3 has changed *Version 10 (53)* - Product description adapted to a new lot (lot number added, new picture included). *Version 09 (49)* - Product description adapted to a new lot (lot number added, new picture included). *Version 08 (48)* - The length of the Exon 8 and 26 probe were adjusted to the actual length. *Version 07 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 06 (48)* - Various minor textual changes. *Version 05 (47)* - Exon numbering of the CAPN3 gene has been changed in Table 1 and 2. - Various minor textual changes on page 1. - Remark on RefSeqGene standard and transcript variant added below Table 2. *Version 04 (46)* - Exon numbering of the CAPN3 gene has been changed on page 3 and 4. - Warning added in Table 1 and 2, 373 nt probe 02531-L01962 and 391 nt probe 10609-L11160. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Data analysis method has been modified. - Various minor textual changes on page 1. - Various minor layout changes.