SALSA MLPA probemix P436-A1 ANO5

Lot A1-1116, A1-0413.

Limb girdle muscular dystrophy type 2L (LGMD2L) or anoctaminopathy is a condition mainly characterised by adult onset proximal lower limb muscular weakness and raised creatine kinase (CK) values, due to recessive anoctamin 5 (ANO5) gene mutations. This gene encodes a member of the anoctamin family of transmembrane proteins, and the encoded protein is likely a calcium activated chloride channel. An exon 5 founder mutation (c.191dupA) represents 61% of mutated alleles and appears to be more prevalent in Northern European populations (Sarkozy et al. 2013 *Hum Mutat)*. c.191dupA mutations lead to a frame shift and to premature truncation, which strongly suggests that c.191dupA is associated with a loss of ANO5 function (Bolduc et al. 2010 *Am J Hum Genet*).

The ANO5 gene (22 exons) ~90 kb of genomic DNA and is located at 11p14.3, 22 Mb from the p-telomere. The P436-A1 probemix contains one probe for every exon, two probes for exon 1, 7 and 19 and three probes for exon 22. Furthermore, the probemix contains a mutation specific probe for c.191dupA which will only generate a signal when the mutation is present. The exon 5 probe detects the wild-type sequence of the c.191dupA mutation, which means that its signal will decrease when the mutation is present. In addition, 10 reference probes are included in this probemix, detecting several different autosomal chromosomal locations.

SD033 Sample DNA: Please note that the mutation-specific probe for c.191dupA has only been tested on control plasmids and not on positive human DNA samples with the c.191dupA mutation! This SD033 sample DNA is provided with each probemix vial and can be used in data binning in the fragment analysis and as a positive control for the mutation-specific probe (see next page).

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned gene, and to detect the presence of the aforementioned c.191dupA mutation 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 this gene are expected to be small (point) mutations 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
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Related SALSA® MLPA® probemixes

- P048 LMNA/MYOT/ZMPSTE24: Contains probes for the MYOT gene, involved in Limb Girdle Muscular Dystrophy 1A (LGMD1A).
- P116 SGC: Contains probes for the SGCA, SGCB, SGCD, SGCG and FKRP genes, involved in various types of Limb Girdle Muscular Dystrophy.
- P176 CAPN3: Contains probes for the CAPN3 gene, involved in Limb Girdle Muscular Dystrophy 2A (LGMD2A).
- P268 DYSF: Contains probes for the DYSF gene, involved in Limb Girdle Muscular Dystrophy 2B (LGMD2B).

Data analysis

The P436-A1 ANO5 probemix contains 38 MLPA probes with amplification products between 136 nt and 400 nt. This includes one probe specific for the c.191dupA mutation which will only generate a signal when the mutation is present. 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.

SD033 Sample DNA

The SD033 Sample DNA provided with this probemix can be used as Binning DNA sample for binning of the c.191dupA mutation-specific probe (ANO5 probe 18658-SP0690-L24012). Inclusion of one reaction with SD033 DNA in MLPA experiments is recommended, as it can be used to aid in data binning of the peak pattern using Coffalyser.NET software, and as an artificial positive control for the specific point mutation.
Please note that SD033 DNA consists of female DNA mixed with a plasmid that contains the target sequence detected by the above mentioned probe + the sequence of the 105 nt chromosome Y specific control fragment. The amount of plasmid used (relative to the genomic DNA) results in a relative probe signal for the 105 nt probe on this female DNA which is identical to the relative probe signal obtained on male DNA samples. As a result, the 100 and 105 nt control fragments indicate the presence of two copies chromosome X and one copy chromosome Y and one copy of the mutation-specific probes (heterozygous mutation). The product description of the SD033 can be found on www.mlpa.com. This product is for research use only.

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 the 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 P436-A1 ANO5

Length (nt)	SALSA MLPA probe	Chromosomal position Reference	Chromosomal position ANO5
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		
136	Reference probe 00797-L24120	5q31	
142	**ANO5 probe** 18651-L24005		Exon 7
148	**ANO5 probe** 18652-L24006		Exon 1
155	**ANO5 probe** 18653-L24007		Exon 16
160	Reference probe 12741-L13835	21q22	
168	**ANO5 probe** 18654-L24797		Exon 1
172	**ANO5 probe** 18655-L24009		Exon 10
177	**ANO5 probe** 18656-L24010		Exon 2
184	**ANO5 probe** 18657-L24011		Exon 17
190	Reference probe 13382-L14839	6q12	
198 § Ж	**ANO5 probe** 18658-SP0690-L24012		**c.191dupA**
204 § Ж	**ANO5 probe** 18658-SP0690-L25311		Exon 5
210	**ANO5 probe** 18659-L25310		Exon 3
219	**ANO5 probe** 18660-L25309		Exon 8
227	**ANO5 probe** 18661-L24015		Exon 12
232	**ANO5 probe** 18662-L24796		Exon 18
238	**ANO5 probe** 18663-L24017		Exon 13
244	**ANO5 probe** 18664-L24018		Exon 22
250	**ANO5 probe** 18665-L24019		Exon 7
256	Reference probe 11349-L12074	12p13	
262	**ANO5 probe** 18666-L24799		Exon 9
267	Reference probe 14110-L15943	8p21	
274	**ANO5 probe** 18667-L24021		Exon 22
281	**ANO5 probe** 18668-L24022		Exon 15
285	**ANO5 probe** 18669-L24023		Exon 4
292	**ANO5 probe** 18670-L24024		Exon 19
301	**ANO5 probe** 18671-L24025		Exon 14
313	Reference probe 06580-L24038	2q24	
319	**ANO5 probe** 18672-L24026		Exon 21
328	**ANO5 probe** 18673-L24027		Exon 6
337	**ANO5 probe** 18674-L24028		Exon 20
346	Reference probe 06015-L07508	19q13	
355	**ANO5 probe** 18675-L24029		Exon 22
364	**ANO5 probe** 18676-L24030		Exon 11
373	Reference probe 00655-L00183	4q27	
384	**ANO5 probe** 18677-L24031		Exon 19
391	Reference probe 07808-L22560	3p22	
400	Reference probe 13588-L24039	1q23	

§ Mutation-specific probe (198 nt). This probe will only generate a signal when the c.191dupA mutation is present. It has been tested on artificial test DNA **but not on positive human samples!** The exon 5 probe (204 nt) detects the wild-type sequence of the c.191dupA mutation. Therefore, the signal will decrease when the mutation is present.
Ж This probe consists of three parts and has two ligation sites.

Note: Exon numbering used here may differ from literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. ANO5 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	ANO5 Exon	Ligation site NM_213599.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*318-320 (exon 1)*		
168	18654-L24797	exon 1	222-223	GGCGGCCCACAG-TCAGATTCAGCA	0.1 kb
148	18652-L24006	exon 1	300-301	GCACCAGTGCCA-TTAACGAGCTGG	10.3 kb
177	18656-L24010	exon 2	383-384	AAGCATATAGAC-TACTCTTTCCAA	7.5 kb
210	18659-L24013	exon 3	408-409	ACTGTTAGCAGA-GCCTGAGCAGCA	7.0 kb
285	18669-L24023	exon 4	495-496	GGCGGCGGCTTA-TGGTAAAACCAG	2.8 kb
198 § Ж	18658-SP0690-L24012	c.191dupA	504-505; 538-539	GCAGTTTCAAAA-34 nt spanning oligo-AGATGGGATTAG	0.0 kb
204 § Ж	18658-SP0690-L25311	exon 5	504-505; 538-539	TGCAGTTTCAAA-34 nt spanning oligo-AGATGGGATTAG	4.9 kb
328	18673-L24027	exon 6	654-653 reverse	TTCCAACTCAAG-ACCTGTTTTTCT	1.3 kb
250	18665-L24019	exon 7	697-698	GGAAGATGGAAG-AACTTATTTTGT	0.1 kb
142	18651-L24005	exon 7	768-769	TGGGAATCAAAA-TGCCTATTAAGG	8.9 kb
219	18660-L24014	exon 8	1059-1060	ACACTTACTCAT-CTGCCTATCCAC	3.3 kb
262	18666-L24799	exon 9	1090-1091	GGGCCAATATTG-GAAGCCATCAGA	10.7 kb
172	18655-L24009	exon 10	1268-1269	CTATTCTTTGCA-GCTGTAGTTGGC	0.4 kb
364	18676-L24030	exon 11	12nt before exon 11	ATAACTTTGCTG-TTCCTCTTGCAG	0.3 kb
227	18661-L24015	exon 12	6nt after exon 12	TTTGGGGTGAGT-AAATAGTCCCAT	4.4 kb
238	18663-L24017	exon 13	1568-1569	CTGGTGGACTTT-GAAGAGGAACAG	2.2 kb
301	18671-L24025	exon 14	15nt before exon 14	TTCTTTGTGATT-TCTTCAATATTA	1.9 kb
281	18668-L24022	exon 15	1775-1776	TACCGCCTGTCA-GTCTTTGCTACA	2.7 kb
155	18653-L24007	exon 16	2075-2076	GTAGGCTATCCT-GGAAAATACACA	0.7 kb
184	18657-L24011	exon 17	2129-2130	TGTGATCCTGGA-GGCTGTCTTATA	7.4 kb
232	18662-L24796	exon 18	2284-2283 reverse	CATGATCCTGCT-CCCATCGACTAT	2.5 kb
292	18670-L24024	exon 19	2416-2417	TGCTCTCATAAA-TAATATTGTAGA	0.1 kb
384	18677-L24031	exon 19	2508-2509	TAGGTGTTTGGC-AAGACATTCTTT	1.7 kb
337	18674-L24028	exon 20	2644-2645	TATGACAGGATA-TGTGAATAATAG	1.5 kb
319	18672-L24026	exon 21	2772-2773	ACGAGAATAAAT-ATTTTCATAATA	3.6 kb
355	18675-L24029	exon 22	3026-3027	ATGATTGAGGAA-AACAAAGCACAG	0.4 kb
244	18664-L24018	exon 22	3376-3377	AGAAACACTGGC-CTTGGGCTGTCC	1.5 kb
274	18667-L24021	exon 22	4876-4877	TGGCTTGTCAAA-TCAGATTCTCCA	-
		stop codon	*3057-3059 (exon 22)*		

§ Mutation-specific probe (198 nt). This probe will only generate a signal when the c.191dupA mutation is present. It has been tested on artificial test DNA **but not on positive human samples!** The exon 5 probe (204 nt) detects the wild-type sequence of the c.191dupA mutation. Therefore, the signal will decrease when the mutation is present.
Ж This probe consists of three parts and has two ligation sites.

WARNING: ANO5 c.191dupA and ANO5 exon 5 probes are designed to target the same location on the genomic DNA. Therefore, the wild type exon 5 amplification probe gives a small signal at 204 nt when a homozygous c.191dupA mutation is present!

Note: The NM_213599.2 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project. The exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com.

SALSA MLPA probemix P436-A1 ANO5 sample pictures

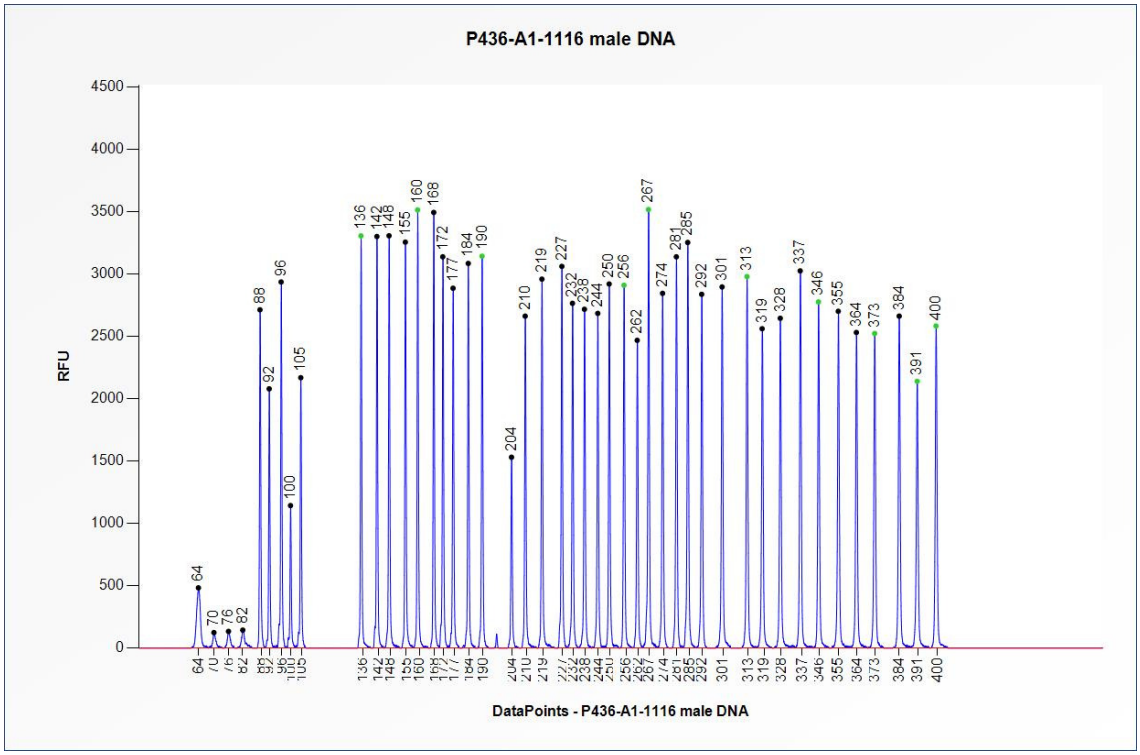

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P436-A1 ANO5 (lot A1-1116).

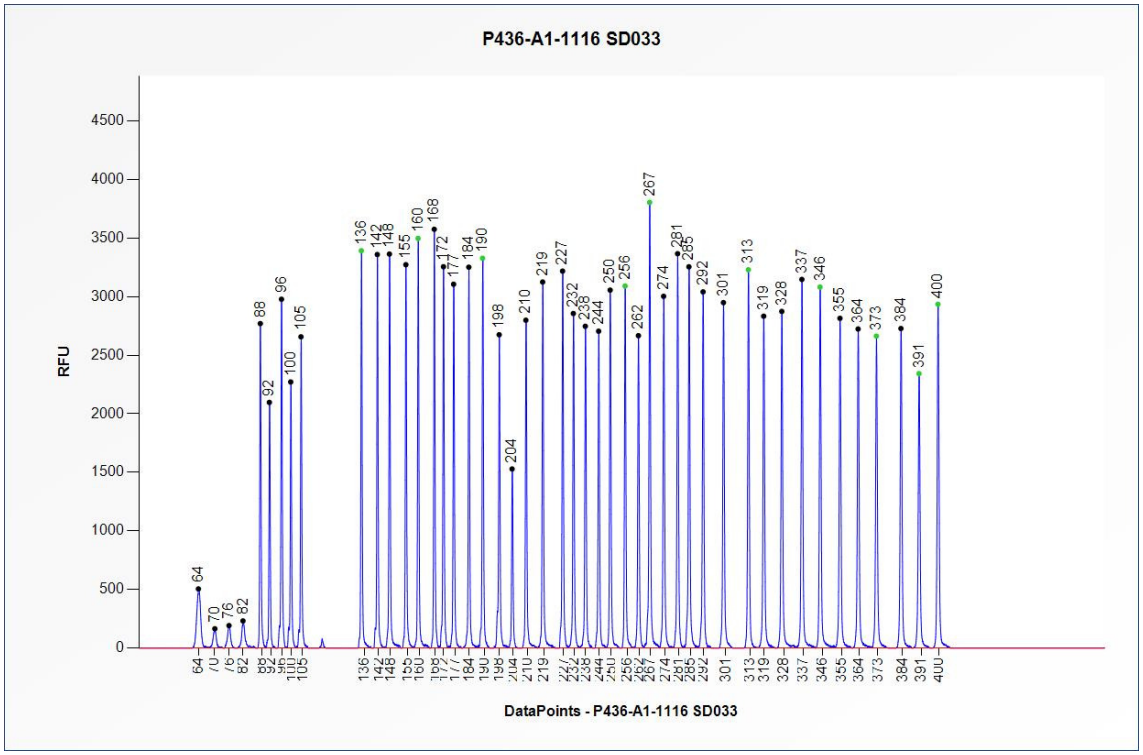

Figure 2. Capillary electrophoresis pattern from an SD033 sample DNA (approximately 50 ng) containing the sequences of the 105 nt chromosome Y specific probe and the c.191dupA analysed with SALSA MLPA probemix P436-A1 ANO5 (lot A1-1116). The location of the c.191dupA mutation specific probe at 198 nt is indicated.

Implemented Changes – compared to the previous product description version(s).
Version 04 – 24 February 2017 (55) - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Manufacturer's address adjusted. *Version 03 – 23 February 2015 (54)* - New sample picture included in product description. - "Peak area" replaced with "peak height". - Updated link for "Database of Genomic Variants". *Version 02 (52)* - Adjusted SD information on page 1 and 2. *Version 01 (50)* - Not applicable, new document.