SALSA MLPA probemix P455-A1 LZTR1

Lot A1-0617, A1-1014

Schwannomatosis (MIM 162091), the third major form of neurofibromatosis, is a late-onset tumour predisposition disorder that is clinically and genetically distinct from neurofibromatosis types 1 (MIM 162200) and 2 (MIM 101000). In approximately 50% of the schwannomatosis cases, germline mutations in SMARCB1 have been identified (Smith et al., *Neurogenetics* 2012). Genetic analysis of the schwannomas showed that mutation of SMARCB1 is often followed by loss of heterozygosity at the 22q region and subsequent mutation of the NF2 gene. Together, these three events result in biallelic loss of the SMARCB1 and NF2 tumour suppressor genes in the schwannomas. In 22q-related schwannomatosis cases without constitutional SMARCB1 mutations, Piotrowski (2014) identified germline mutations in LZTR1 (Piotrowski et al., *Nat Genet* 2014). Mutations in LZTR1 may account for up to 80% of the schwannomatosis cases lacking mutations in SMARCB1.

The LZTR1 gene (21 exons) spans ~16.8 kb of genomic DNA and is located on 22q11.21, 20 Mb from the p-telomere. The P455-A1 probemix contains one probe for each exon of the gene with the exception of exon 4 and 17. Two probes are included for exon 21. This probemix furthermore contains 11 flanking probes targeting the 22q11 and 22q12 chromosomal regions surrounding LZTR1. In addition, 14 reference probes are included in this probemix, 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(s) 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

- P044 NF2: Contains probes for the NF2 gene, involved in Neurofibromatosis type 2.
- P081/P082 NF1: Contains probes for the NF1 gene, involved in Neurofibromatosis type 1.
- P250 DiGeorge: Contains probes for the 22q11 region; used for primary screening of this region.
- P258 SMARCB1: Contains probes for the SMARCB1 gene; deletions and mutations in SMARCB1 are associated with malignant rhabdoid tumours and schwannomatosis.
- P295 SPRED1: Contains probes for the SPRED1 gene, involved in Neurofibromatosis type I-like syndrome.

Data analysis

The P455-A1 LZTR1 probemix contains 45 MLPA probes with amplification products between 124 and 500 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 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 P455-A1 LZTR1 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position		
		reference	LZTR1	flanking
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			
124	Reference probe 18709-L21056	5q31		
131	Reference probe 16316-L22397	3q21		
136	Reference probe 10995-L11666	4q22		
153 ¬	**THAP7 probe** 20047-L27123			Exon 1
161 ¬	**KLHL22 probe** 20041-L05815			Exon 2
166	**LZTR1 probe** 19994-L27073		**Exon 6**	
172	**LZTR1 probe** 19995-L27440		**Exon 10**	
178	**LZTR1 probe** 19996-L27075		**Exon 15**	
184 ¬	**RIMBP3C probe** 20042-L05796			Upstream
190	Reference probe 08838-L08898	2p13		
196	Reference probe 18049-L22439	16q23		
202	**LZTR1 probe** 19997-L27076		**Exon 2**	
214	**LZTR1 probe** 19999-L27078		**Exon 8**	
220 Ж	**LZTR1 probe** 20000-SP0909-L27079		**Exon 21**	
226 ¬	**CRKL probe** 20043-L20997			Exon 1
232	Reference probe 16428-L25931	18q21		
244	**LZTR1 probe** 20001-L27442		**Exon 19**	
250	**LZTR1 probe** 20002-L27081		**Exon 12**	
256 Ж	**LZTR1 probe** 20003-SP0910-L27443		**Exon 21**	
265	**LZTR1 probe** 20004-L27083		**Exon 1**	
275 ¬	**SNAP29 probe** 20044-L23315			Exon 3
280	Reference probe 13350-L26120	9q21		
288	Reference probe 13393-L26813	6q12		
301 Ж	**LZTR1 probe** 20005-SP0911-L27084		**Exon 5**	
310	**LZTR1 probe** 20006-L27085		**Exon 11**	
318	**LZTR1 probe** 20007-L27086		**Exon 20**	
326	**LZTR1 probe** 20008-L27087		**Exon 13**	
334 Ж ¬	**THAP7 probe** 20048-SP0915-L27124			Exon 1
346 ¬	**MYH9 probe** 18630-L23987			Exon 13
355	Reference probe 05991-L05416	20p12		
364	**LZTR1 probe** 20009-L27088		**Exon 7**	
373	**LZTR1 probe** 20010-L27089		**Exon 18**	
390 Ж	**LZTR1 probe** 20012-SP0912-L27750		**Exon 14**	
400 ¬	**CRKL probe** 20045-L16112			Exon 3
408	Reference probe 08906-L20708	11p11		
417	Reference probe 13817-L15311	2q13		
427 ¬	**HIC2 probe** 20046-L15009			Exon 2
436	**LZTR1 probe** 20013-L27092		**Exon 9**	
444 Ж	**LZTR1 probe** 20014-SP0913-L27093		**Exon 16**	
454	**LZTR1 probe** 20015-L27094		**Exon 3**	
463 ¬	**THAP7 probe** 20049-L27125			Exon 3
474 ¬	**LARGE probe** 12461-L13462			Exon 3
481	Reference probe 14909-L05359	18p11		
494	Reference probe 19137-L26747	21q22		
500	Reference probe 19675-L26275	4p13		

Ж This probe consists of three parts and has two ligation sites.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

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. P455 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	LZTR1 Exon	Ligation site NM_006767.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
161 ¬	20041-L05815	KLHL22		TCTTCGATGTTG-TGCTGGTGGTGG	392.1 kb
275 ¬	20044-L23315	SNAP29		GTATCCACTTAC-CTGTATCATCCA	36.8 kb
226 ¬	20043-L20997	CRKL ex 1		GGTTCGACTCCT-CGGACCGCTCCG	32.0 kb
400 ¬	20045-L16112	CRKL ex 3		ATTGCCGAAGTC-CAGCTTTCTGCA	32.6 kb
		start codon	*104-106 (exon 1)*		
265	20004-L27083	Exon 1	285-286	CCCGCCCTGCGA-CGAGTTCGTGGG	0.5 kb
202	19997-L27076	Exon 2	344-345	AAGATGCCATTT-ATGTATTTGGTG	2.8 kb
454	20015-L27094	Exon 3	387-388	GCTCAATGACCT-CCTGCGGTTCGA	2.3 kb
	No Probe	*Exon 4*			
301 Ж	20005-SP0911-L27084	Exon 5	1 nt; 34 nt after exon 5	ATTGAAGGACGG-33nt spanning oligo-GGGTCCTGGGTG	0.7 kb
166	19994-L27073	Exon 6	631-632	GTCGCTAGGTCA-GCCCATGGGGCC	0.8 kb
364	20009-L27088	Exon 7	14 nt before exon 7	GTCCTCACTGGT-CTGTCCTAATAC	0.8 kb
214	19999-L27078	Exon 8	792-793	ATCTTGCTGCAA-CTTCCCCGTGGC	1.2 kb
436	20013-L27092	Exon 9	896-897	GTACCCCCAGGT-GGACACGCATCC	0.7 kb
172	19995-L27440	Exon 10	1252-intron 10	CCTGCCTCGGAG-GTACAGGCTGGG	0.5 kb
310	20006-L27085	Exon 11	1297-1298	GTCATCTCGGAC-GCCATGTACATC	0.8 kb
250	20002-L27081	Exon 12	1378-1379	TCCTGTTACCCT-AAATGCACGCTG	0.3 kb
326	20008-L27087	Exon 13	1483-1484	CAGGGCCACGTA-GCCATTGTCACA	0.1 kb
390 Ж	20012-SP0912-L27750	Exon 14	17 nt before exon 14; 1562-1563	GGTGTCCTTGAG-27nt spanning oligo-AGGAGGCCGCCC	0.5 kb
178	19996-L27075	Exon 15	1769-1770	AACTGGCACTGA-GCTTCCAGTTGT	0.3 kb
444 Ж	20014-SP0913-L27093	Exon 16	1911-1912; 1950-1951	GAACTTCGTGGT-39nt spanning oligo-GTTCGAGCGCCT	1.1 kb
	No Probe	*Exon 17*			
373	20010-L27089	Exon 18	2178-2179	CTGCAGCTACTT-TGAAGCCATGTT	0.7 kb
244	20001-L27442	Exon 19	2331-2332	CAGCTACTTGTT-TGCGGCCCCCTA	0.3 kb
318	20007-L27086	Exon 20	2500-2501	ATTGTGCACCAG-TTCACCAAGGTC	0.5 kb
220 Ж	20000-SP0909-L27079	Exon 21	2733-2734; 2760-2761	GTGCACCTGCCA-27nt spanning oligo-CCAAAGAGAGCT	1.4 kb
256 Ж	20003-SP0910-L27443	Exon 21	4148-4149; 4174-4175	TGGCTCCTGCCA-26nt spanning oligo-CCCTCCTGGAGG	1.9 kb
		stop codon	*2624-2626 (exon 21)*		
463 ¬	20049-L27125	THAP7 ex 3		CTTTCTCCAAGT-TGCGCCGGACAA	1.2 kb
153 ¬	20047-L27123	THAP7 ex 1		TCTTTCCCAGAT-GCCGCGTCACTG	0.2 kb
334 Ж ¬	20048-SP0915-L27124	THAP7 ex 1		CGCAGCAATGGC-29nt spanning oligo-AGAGAATCGGCT	443.1 kb
427 ¬	20046-L15009	HIC2		GTTCCAGCAGAT-CTTGGACTTCAT	117.7 kb
184 ¬	20042-L05796	RIMBP3C		CTGGGCCCAAGG-CCTAATAGGTGA	12240.4 kb
474 ¬	12461-L13462	LARGE		ATCTCCAGTGCT-AAGAACTCAGGC	2552.7 kb
346 ¬	18630-L23987	MYH9		CAGCTGAACTCG-TTTGAGCAGCTG	

Ж This probe consists of three parts and has two ligation sites.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.

The NM_006767.3 sequence is a reference standard in the NCBI RefSeqGene project.

Note: Exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P455-A1 LZTR1 sample picture

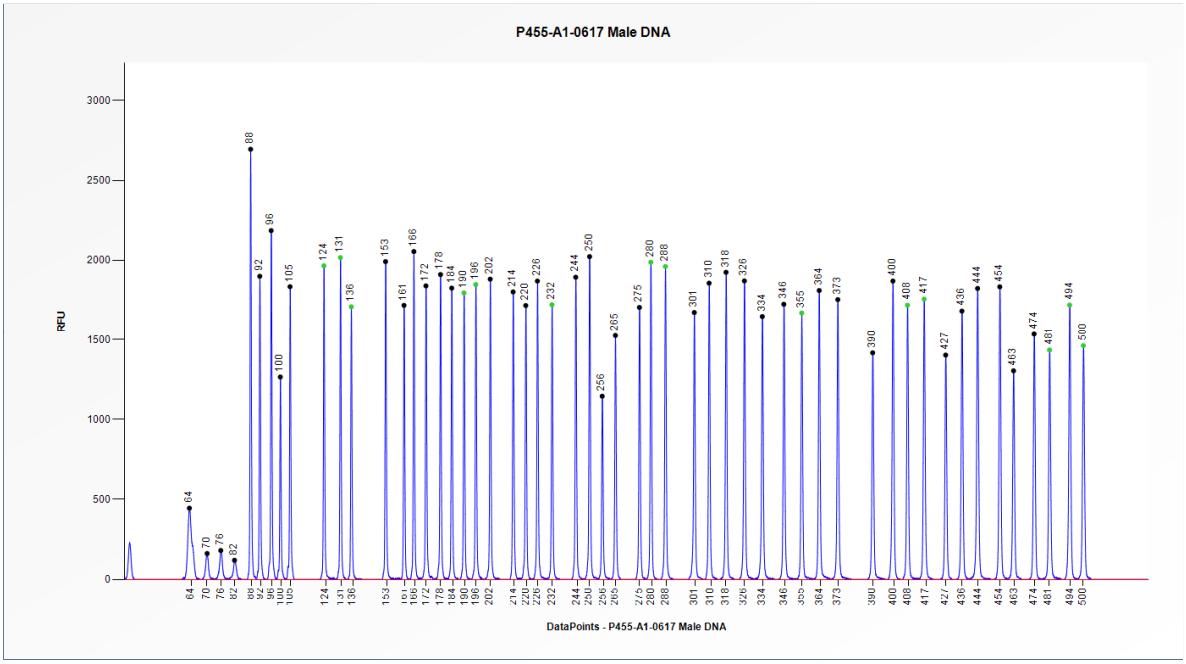

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P455-A1 LZTR1 (lot A1-0617).

Implemented Changes – compared to the previous product description version.
Version 02 – 12 October 2017 (55) - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). *Version 01 – 05 February 2015 (54)* - Not applicable, new document.