SALSA MLPA probemix P395-A2 MEF2C-FOXG1

Lot A2-0217, lot A2-1113. As compared to the previous version (lot A1-0711), three probes have been elongated.

Mental retardation can be provoked by different chromosomal aberrations for example deletions of the chromosomal regions 5q14.3 and 14q12. Deletions of the 5q14.3 region mainly affect MEF2C (myocyte enhancer factor 2C), and it was shown that microdeletions in this gene are involved in severe mental retardation, stereotypic movements, epilepsy, and/or cerebral malformation (Zweier et al. 2010; Le Meur et al. 2010; Nowakowska et al. 2010). Another gene that has been implicated in mental retardation is the FOXG1 (forkhead box G1) gene. Its deletion could be linked to a congenital variant of Rett syndrome (Mencarelli et al. 2009; Kortüm et al. 2011).

The MEF2C gene (11 exons) spans ~186 kb of genomic DNA and is located on chromosome 5q14.3, ~88 Mb from the p-telomere. This P395-A2 probemix contains probes for each exon of MEF2C. Furthermore: also, one additional probe for exon 4 and 11; two for exon 5; two for intron 2; one for introns 3, 7, 8 are included in this probemix. Furthermore, the probemix contains four flanking probes for MEF2C. Two are located downstream in the TMEM161B and RASA1 genes and two upstream in the GPR98 and CETN3 genes.

The FOXG1 gene (1 exon) spans ~3.2 kb of genomic DNA and is located on chromosome 14q12, ~28 Mb form the p-telomere. This P395-A2 probemix contains three probes for exon 1 and two probes upstream of exon 1. In addition, 11 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).

Related SALSA® MLPA® probemixes

- P064 Microdeletion Syndromes-1B: Contains probes for 1p-deletion syndrome, Williams syndrome, Smith-Magenis syndrome, Miller-Dieker syndrome, DiGeorge syndrome, Prader-Willi syndrome, Alagille syndrome, Saethre-Chotzen syndrome and Sotos syndrome.
- P106 MRX: Contains probes for various genes involved in X-linked mental retardation.
- P245 Microdeletion Syndromes-1A: Contains probes for 21 different microdeletion syndromes and can be used for primary screening of microdeletion syndromes.
- P036 Subtelomere Mix 1 : Contains a probe for every human subtelomere.
- P070 Subtelomere Mix 2B: Contains a probe for every human subtelomere. Can be used as confirmation probemix of P036 Subtelomere Mix 1.

More information

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Data analysis

The P395-A2 MEF2C-FOXG1 probemix contains 40 MLPA probes with amplification products between 129 and 472 nt. 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 normalized 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 normalization). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalized probe ratio in a sample by the average intra-normalized probe ratio of all reference samples. Please note that this type of normalization 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 P395-A2 MEF2C-FOXG1 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position		
		reference	MEF2C	FOXG1
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			
129	Reference probe 11622-L12379	10q25		
136	**MEF2C probe** 16495-L18956		**Exon 11**	
142	Reference probe 07721-L07431	7p13		
148 ¬	**TMEM161B probe** 17287-L20747		**Upstream**	
154	**MEF2C probe** 16496-L18957		**Exon 2**	
160	**MEF2C probe** 16497-L18958		**Exon 5**	
166	Reference probe 10671-L11253	6p12		
172	**MEF2C probe** 16498-L18959		**Intron 8**	
178	**MEF2C probe** 16499-L18960		**Exon 5**	
184	Reference probe 03269-L13647	3q29		
190	**MEF2C probe** 16500-L18961		**Exon 4**	
196 ±	**FOXG1 probe** 13756-L20771			**Upstream**
202	**MEF2C probe** 16502-L20741		**Exon 8**	
208 ¬	**RASA1 probe** 16501-L20775		**Upstream**	
214	**MEF2C probe** 16503-L18964		**Exon 4**	
228 ¥	**MEF2C probe** 16504-L26470		**Exon 9**	
235 ¥	**MEF2C probe** 16505-L26471		**Exon 3**	
241 ¥	Reference probe 07909-L26472	17q23		
247	**MEF2C probe** 16506-L18967		**Exon 5**	
256 ¬	**GPR98 probe** 17288-L20748		**Downstream**	
265 Ж	**MEF2C probe** 17289-SP0470-L20749		**Exon 1**	
274	**MEF2C probe** 16507-L18968		**Intron 2**	
283 ±	**FOXG1 probe** 13755-L20772			**Exon 1**
292	Reference probe 12495-L13539	1q32		
301 ±	**FOXG1 probe** 13757-L15244			**Exon 1**
310	**MEF2C probe** 16509-L18970		**Exon 6**	
319	Reference probe 13345-L14771	18q21		
337	**MEF2C probe** 16510-L20742		**Intron 7**	
344 ±	**FOXG1 probe** 16850-L20773			**Upstream**
355	**MEF2C probe** 16511-L18972		**Exon 10**	
364 ‡	**MEF2C probe** 16512-L18973		**Intron 3**	
382	**MEF2C probe** 16513-L18974		**Exon 11**	
391 ¬	**CETN3 probe** 16514-L18975		**Downstream**	
400 ‡	Reference probe 12260-L14061	12q23		
409	**MEF2C probe** 16515-L18976		**Intron 2**	
418	**MEF2C probe** 16516-L18977		**Exon 7**	
427	Reference probe 04444-L03830	8p21		
454	Reference probe 08479-L08490	22q11		
463 ±	**FOXG1 probe** 13754-L15241			**Exon 1**
472	Reference probe 12761-L13877	4q12		

¥ Changed in version A2 (from lot A2-1113 onwards). Small change in length, no change in sequence detected.
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.
‡ SNP rs116418914 (364 nt), rs111842675 (400 nt) and rs79643666 (400 nt) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
± These probes are located within, or close to, a very strong CpG island. A low signal of these probes can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

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.

Table 2. P395 probes arranged according to chromosomal location

Table 2a. MEF2C

Length (nt)	SALSA MLPA probe	MEF2C Exon	Ligation site NM_001131005.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
256 ¬	17288-L20748		GPR98 gene	CTCATAATTCCA-GTAGTTCGTGGA	217.7 kb
391 ¬	16514-L18975		CETN3 gene	TTACCGACCTCA-GAGCTAAACTCA	1505.2 kb
		Start codon	*430-432 (ex 3)*		
265 Ж	17289-SP0470-L20749	Exon 1	450 nt; 407 nt before ex 1	AATGAGCTGCAA-43nt spanning oligo-CCTGAAATGAAG	16.9 kb
154	16496-L18957	Exon 2	225-224 reverse	TCTCTTCATAGA-AGTTACTTCACA	0.6 kb
274	16507-L18968	Intron 2	582 nt after ex 2 reverse	CTTCCCTACATT-AAAGCAGCGGTC	3.8 kb
409	16515-L18976	Intron 2	4356 nt after ex 2	TACACCCAGACA-TCTTCGGGCTGC	59.2 kb
235 ¥	16505-L26471	Exon 3	321-322	GGAACTGAGCTG-TGCAAGTGCTGA	1.1 kb
364 ‡	16512-L18973	Intron 3	897 nt after ex 3	TGCAGAGAAGAC-GTTTCTGGACTG	18.1 kb
190	16500-L18961	Exon 4	564-565	GAGATTGCGCTG-ATCATCTTCAAC	0.1 kb
214	16503-L18964	Exon 4	4 nt after ex 4	TCGTGGAGGTGA-GAGAGCATGCGT	43.0 kb
160	16497-L18958	Exon 5	447 nt before ex 5	CATGAAACTTGA-GTCCTTATACCC	0.2 kb
247	16506-L18967	Exon 5	200 nt before ex 5	GTATTTCAGACG-TTGAGAAAGAAG	0.4 kb
178	16499-L18960	Exon 5	18 nt after ex 5	ACCAAAGGTAGA-TGGCTGGTCTGC	9.1 kb
310	16509-L18970	Exon 6	981-982	TCTCCTGGTGTA-ACACATCGACCT	2.8 kb
418	16516-L18977	Exon 7	1036-1037	GTGGAGACCTCA-CGTCTGGTGCAG	16.5 kb
337	16510-L20742	Intron 7	679 nt before ex 8	TGCCTTGGTGAA-TCAGCACTTTAG	0.8 kb
202	16502-L20741	Exon 8	1195-1196	ATCTCCGAGTTC-TTATTCCACCAG	1.5 kb
172	16498-L18959	Intron 8	879 nt before ex 9	TCAGTCTGAGGA-TGTCGACCTGCT	0.9 kb
228 ¥	16504-L26470	Exon 9	1269-1270	TCGGCTCAGTCA-TTGGCTACCCCA	0.7 kb
355	16511-L18972	Exon 10	1392-1393	GCAGACCTGTCA-TCTCTGTCTGGG	5.7 kb
382	16513-L18974	Exon 11	1528-1529	ATTTATCTCAGA-GTTCAAATCTCT	0.1 kb
136	16495-L18956	Exon 11	1689-1688 reverse	CTCCCGTCGTAC-GAACTGCTACAG	526.8 kb
		Stop codon	*1819-1821 (ex 11)*		
148 ¬	17287-L20747		TMEM161B gene	GGTTTCTGTAAA-CCAGGGTAATCA	864.5 kb
208 ¬	16501-L20775		RASA1 gene	AGAAGAACGCCT-CAGGCAGGCAGG	

¥ Changed in version A2 (from lot A2-1113 onwards). Small change in length, no change in sequence detected.

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.

‡ SNP rs116418914 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

± These probes are located within, or close to, a very strong CpG island. A low signal of these probes can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

The NM_001131005.2 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2b. FOXG1

Length (nt)	SALSA MLPA probe	FOXG1 Exon	Ligation site NM_005249.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
344 ±	16850-L20773	Upstream	844 nt before ex 1	AAATGCCAGACA-CTGGCCTGCAAG	0.2 kb
196 ±	13756-L20771	Upstream	634 nt before ex 1	GAGGAAGCCGGA-AATGTGAGCTAT	0.9 kb
		Start codon	*209-211 (ex 1)*		
301 ±	13757-L15244	Exon 1	240-241	GAAAGAGGTGAA-AATGATCCCCAA	1.4 kb
283 ±	13755-L20772	Exon 1	1622-1621 reverse	GAAATAATCAGA-CAGTCCCCCAGA	0.2 kb
463 ±	13754-L15241	Exon 1	1816-1817	TCTAGGGTTGTT-TATTATTCTAAC	
		Stop codon	*1676-1678 (ex 1)*		

± These probes are located within, or close to, a very strong CpG island. A low signal of these probes can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

The NM_005249.4 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 P395-A2 MEFC2-FOXG1 sample picture

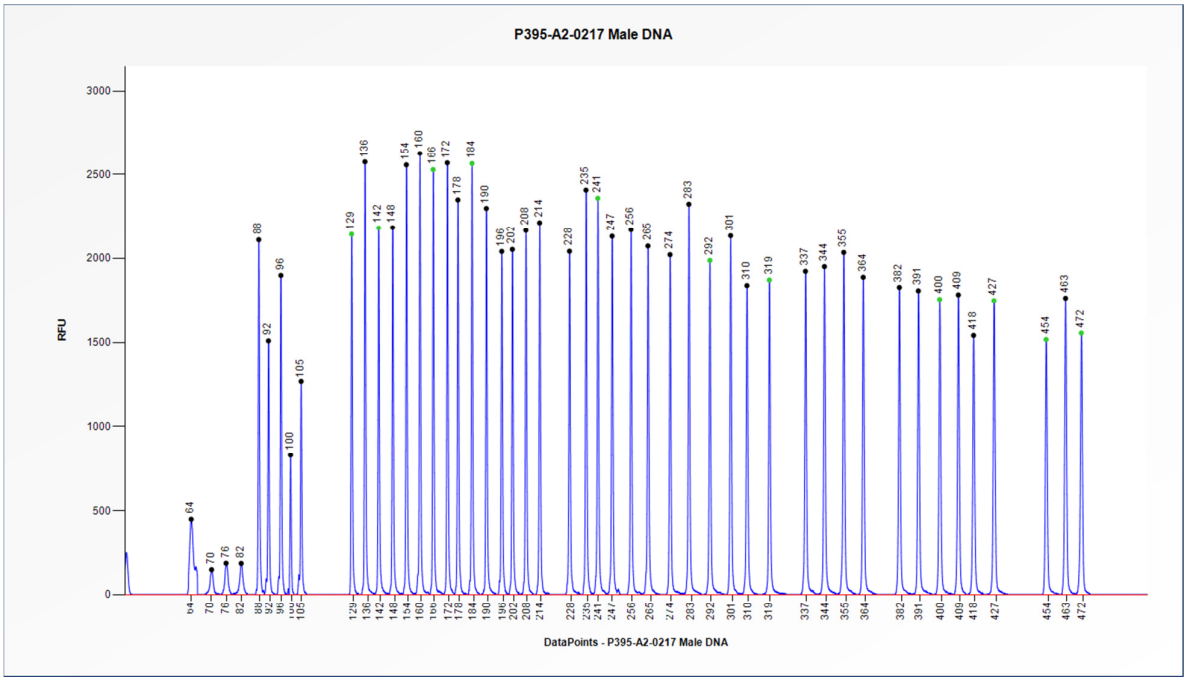

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P395-A2 MEF2C-FOXG1 (lot A2-0217) using Coffalyser.Net.

Implemented Changes – compared to the previous product description version(s).
Version 04 – 12 September 2017 (55) – Product description adapted to a new product lot (lot number changed, changes in Table 1 and Table 2, new picture included). *Version 03 (53)* – For the MEF2C gene; exon numbers in table 1 and 2, ligation sites in table 2 have been adjusted according to NCBI Map Viewer. For the FOXG1 gene; ligation sites in table 2 have been adjusted. – Product description adapted to a new product version (lot number added, new picture included). – Minor textual changes on page 1 and 2. – Changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. *Version 02 (48)* – Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 01 (48)* – Not applicable, new document.