SALSA MLPA probemix P106-C1 MRX

Lot C1-0416. As compared to the previous lot B2-0812, one ARX probe has been replaced. The Y-chromosome fragment on 118 nt has been elongated to 121 nt.

Mental retardation (MR) is defined as a significant impairment of cognitive and adaptive functioning, with onset before age 18 years, and it is estimated to occur in about 1-3% of the population (Chelly and Mandel, 2001, *Nat Rev Genet*.). Among mentally retarded patients, an excess of males over females has long been noted, which is usually explained by the presence of many genes responsible for MR on the X chromosome.

X-linked mental retardation (XLMR) is usually divided into syndromic and non-syndromic or non-specific forms. In syndromic forms (MRXS), MR is present in association with a specific pattern of physical, neurological, and/or metabolic abnormalities. The term non-specific or non-syndromic X-linked mental retardation (MRX) was introduced to indicate a condition segregating in an X-linked manner in which male patients have no consistent phenotypic manifestations other than MR. Many different genes responsible for MRX have been identified.

This P106-C1 MRX MLPA probemix can be used to detect copy number changes of several genes on the X-chromosome that have been implicated in (non-specific) X-linked mental retardation. The MLPA P106-C1 MRX probemix includes probes for 16 different MRX genes: RPS6KA3, ARX, IL1RAPL1, TSPAN7, PQBP1, HUWE1, OPHN1, ACSL4, PAK3, DCX, AGTR2, ARHGEF6, FMR1, AFF2 (FMR2), SLC6A8 and GDI1. For most genes, probes are present for only some of the exons.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned genes in a DNA sample. In males, deletions of a probe's recognition sequence on the X-chromosome will lead to a complete absence of the corresponding probe amplification product, whereas female heterozygotes are recognisable by a 35-50% reduction in relative peak height. 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.

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.

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

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Related SALSA MLPA probemixes

- P245 Microdeletion-1: Probes are included for 21 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
- P036 Subtelomeres Mix 1 / P070 Subtelomeres Mix 2B: These probemixes each contain one probe for every subtelomere.
- More probemixes for specific subtelomere analysis are available. See www.mlpa.com.
- P064 MR-1 and P096 MR-2: Contain probes for several mental retardation syndromes.
- Several syndrome-specific probemixes useful for confirmation of findings with this P106 MRX probemix are available; see page 4-7.

References for SALSA MLPA probemix P106 MRX

- Utine, G.E. et al., 2012. Searching for copy number changes in nonsyndromic X-linked intellectual disability. *Mol Syndromol.* 2:64-71.
- Flynn, M. et al., 2011. Whole gene duplication of the PQBP1 gene in syndrome resembling Renpenning. *Am J Med Genet. A.* 155A:141-4.
- Madrigal, I. et al., 2007. MLPA as first screening method for the detection of microduplications and microdeletions in patients with X-linked mental retardation. *Genet Med*. 9:117-22.
- Tejada, M. et al., 2011. A child with mild X-linked intellectual disability and a microduplication at Xp22.12 including RPS6KA3. *Pediatrics.* 128:e1029

Data analysis

The P106-C1 MRX probemix contains 46 MLPA probes with amplification products between 130 and 481 nt. In addition, it contains 10 control fragments generating an amplification product smaller than 122 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 two Y-fragments at 105 nt and 121 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix should be normalised with a more robust method. (1) Intra-sample normalisation should be performed by dividing the signal of each probe by the signal of every other probe in that sample, thus creating as many ratios per probe as there are other probes. Subsequently, the median of all these produced ratios per probe should be taken; this is the probe's Normalisation Constant. (2) Secondly, inter-sample comparison should be performed by dividing the Normalisation Constant of each probe in a given sample by the average Normalisation Constant of that probe in all the reference samples.

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.

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

Table 1. SALSA MLPA P106-C1 MRX probemix

Length (nt)	SALSA MLPA probe	Chromosomal position
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	
121 ¥	Y-fragment: Specific for the Y chromosome	
130	**AGTR2 probe** 13917-L02320	Xq23
136 ±	**GDI1 probe** 16875-L19669	Xq28
142	**FMR1 probe** 02928-L03720	Xq27
148 ±	**ACSL4 probe** 02935-L02326	Xq23
154 ±	**AFF2 probe** 03511-L04202	Xq28
160 ±	**TSPAN7 probe** 02903-L02297	Xp11
166	**FMR1 probe** 02927-L03721	Xq27
172	**ARHGEF6 probe** 16857-L19651	Xq26
178	**RPS6KA3 probe** 02907-L02301	Xp22
184	**HUWE1 probe** 13919-L15456	Xp11
190 ~ ±	**ACSL4 probe** 02155-L15826	Xq23
195 ± *	**ARX probe** 18790-L24221	Xp21
202	**ARHGEF6 probe** 02902-L04460	Xq26
208 ±	**SLC6A8 probe** 01871-L15827	Xq28
215	**DCX probe** 04123-L15828	Xq23
222 ±	**ARX probe** 02898-L04200	Xp21
229 ±	**ARX probe** 13669-L15822	Xp21
235	**IL1RAPL1 probe** 02922-L23556	Xp21
241	**AFF2 probe** 03516-L15823	Xq28
248	**ACSL4 probe** 03512-L23557	Xq23
256	**HUWE1 probe** 13920-L23672	Xp11
263	**AFF2 probe** 02933-L23673	Xq28
268 ~	**TSPAN7 probe** 02904-L23558	Xp11
275	**DCX probe** 04124-L03481	Xq23
283	**AFF2 probe** 00493-L00066	Xq28
292	**IL1RAPL1 probe** 02920-L02314	Xp21
301 ±	**SLC6A8 probe** 01876-L01445	Xq28
310	**PQBP1 probe** 02918-L02878	Xp11
319	**DCX probe** 04121-L08390	Xq23
328	**IL1RAPL1 probe** 02921-L02315	Xp21
337	**AFF2 probe** 02932-L02323	Xq28
346	**ARHGEF6 probe** 03719-L02293	Xq26
355	**AGTR2 probe** 02925-L02319	Xq23
364	**RPS6KA3 probe** 02906-L02300	Xp22
371	**OPHN1 probe** 02912-L02306	Xq12
378 ±	**GDI1 probe** 16874-L23559	Xq28
385	**PAK3 probe** 02908-L03178	Xq23
391	**PQBP1 probe** 03520-L02313	Xp11
400	**PAK3 probe** 03521-L02304	Xq23
409	**OPHN1 probe** 02913-L23560	Xq12
418	**PAK3 probe** 02909-L02303	Xq23
427	**IL1RAPL1 probe** 02923-L23561	Xp21
436	**OPHN1 probe** 02914-L02308	Xq12
443	**ARHGEF6 probe** 16856-L19650	Xq26
472	**OPHN1 probe** 02915-L02309	Xq12
481	**PAK3 probe** 02911-L02305	Xq23

* New in version C1 (from lot C1-0416 onwards).
¥ Changed in version C1 (from lot C1-0416 onwards). Small change in length, no change in hybridising sequence.
± 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.
~ More variable. This probe has been reported to be deleted/duplicated in healthy individuals (various reports).

Table 2. P106 probes arranged according to chromosomal location

Table 2a. RPS6KA3 gene, Xp22.12

Length (nt)	SALSA MLPA probe	RPS6KA3 Exon	Ligation site NM_004586.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2221-2223 (ex 22)*		
178	02907-L02301	Exon 21	2005-2006	ATCAGAGACTGA-CTGCTGCTCTTG	53.1 kb
364	02906-L02300	Exon 3	180-181	AAGGAAGGACAT-GAAAAGGCAGAT	4797 kb to ARX gene
		start codon	*1-3 (ex 1)*		

Some mutations in the RPS6KA3 gene, spanning 110 kb, cause mild mental retardation. Most mutations (incl. truncating) cause Coffin-Lowry syndrome. Coffin-Lowry syndrome is characterised by mental retardation and strange fingers, large ears, etc.

- P259 Coffin-Lowry: contains more probes for RPS6KA3.

The NM_004586.2 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2b. ARX gene, Xp21.3

Length (nt)	SALSA MLPA probe	ARX Exon	Ligation site NM_139058.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*1898-1900 (ex 5)*		
222	02898-L04200	Exon 4	1635-1634 reverse	GGCTGATGAAAG-CTGGGTGTCGGA	6.2 kb
195 *	18790-L24221	Exon 2	1151-1150 reverse	GCTGCCCGCAGA-GAGGCACACGCT	2.5 kb
229	13669-L15822	Exon 1	98-99	AGATCGCAATAA-TATCCGTTATAA	3572 kb to IL1RAPL1 gene
		start codon	*212-214 (ex 1)*		

* New in version C1.

Disruption/mutations in the ARX (11 kb) gene cause severe X-linked infantile spasms and mental retardation.

- P189 RETT like: contains more probes for the ARX gene.

The NM_139058.2 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2c. IL1RAPL1 gene, Xp21.3

Length (nt)	SALSA MLPA probe	IL1RAPL1 Exon	Ligation site NM_014271.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*509-511 (ex 2)*		
292	02920-L02314	Exon 1	245-246	CAGCAAACAATC-GGGCACTTTGAG	201.5 kb
328	02921-L02315	Exon 2	518-519	AGATGAAAGCTC-CGATTCCACACT	493.7 kb
235	02922-L23556	Exon 3	716-717	TTGCCCAAAGTG-CTGGACTCAGTT	385.4 kb
427	02923-L23561	Exon 6	1226-1227	CTCTGACTGATA-AGCCACCCAAGC	8709 kb to TSPAN7 gene
		stop codon	*2597-2599 (ex 11)*		

Truncating mutations of IL1RAPL1 have been identified in families with X-linked non-syndromic mental retardation (MRX21). The IL1RAPL1 gene is 1370 kb long.

The NM_014271.3 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2d. TSPAN7 (TM4SF2) gene, Xp11.4

Length (nt)	SALSA MLPA probe	TSPAN7 Exon	Ligation site NM_004615.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*70-72 (ex 1)*		
160	02903-L02297	Exon 1	96-97	ATGGAGACCAAA-CCTGTGATAACC	114.1 kb
268	02904-23558	Exon 5	531-532	TGTGGTGTGCAG-AACTACACCAAC	10221 kb to PQBP1 gene
		stop codon	*817-819 (ex 7)*		

Please note that the last exons of TSPAN7 are in a region that is duplicated in some healthy individuals (variation 34415 of the TCAG database of genomic variants; frequency approximately 1:500). As a result, the 268 nt probe may show an increased signal in healthy persons. This duplication apparently does not disrupt the gene. Truncating mutations in this gene have been identified as the cause of mental retardation (MRX58). The TSPAN7 gene is 125 kb long.

The NM_004615.3 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2e. PQBP1 gene, Xp11.23

Length (nt)	SALSA MLPA probe	PQBP1 Exon	Ligation site NM_005710.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*255-257 (ex 1)*		
310	02918-L02878	Exon 1	155-156	AGATGAGTACAT-GTTTACGGGAGG	3.8 kb
391	03520-L02313	Exon 4	567-568	AAAAGTTGGACC-GGAGCCATGACA	4954 kb to HUWE1 gene
		stop codon	*1050-1052 (ex 6)*		

A variable phenotype of X-linked mental retardation is caused by mutation in the gene encoding the polyglutamine-binding protein-1 (PQBP1). Changes in an AG6 repeat in exon 5 of the PQBP1 gene causes mental retardation. This gene is only 5 kb long.

- P259 Coffin-Lowry: contains more probes for PQBP1 gene.

The NM_005710.2 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Table 2f. HUWE1 gene, Xp11.22

Length (nt)	SALSA MLPA probe	HUWE1 Exon	Ligation site NM_031407.5	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*13551-13553 (ex 84)*		
184	13919-L15456	Exon 61	8702-8703	ATCTGAGTCCAA-GGAGACCCTTGG	92.6 kb
256	13920-L23672	Exon 6	633-634	GCAGATGCTGGA-CAGACAGTGGAG	13510 kb to OPHN1 gene
		start codon	*427-429 (ex 4)*		

The total length of the HUWE1 gene is 155 kb. Duplications of the HUWE1 gene are associated with mental retardation: Froyen, G. et al., 2008, *Am J Hum Genet.* One large deletion is described in a "control sample" in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home.

The NM_031407.5 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2g. OPHN1 gene, Xq12

Length (nt)	SALSA MLPA probe	OPHN1 Exon	Ligation site NM_002547.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2701-2703 (ex 24)*		
472	02915-L02309	Exon 21	2171-2172	TATCACCAGCAG-CATAGAACCCCC	133.1 kb
436	02914-L02308	Exon 12	1351-1352	AGGCCCTTTCAG-AAGCTAACAGAA	101.8 kb
409	02913-L23560	Exon 3	489-490	CAGACGCTGCAG-TCATTTCAGTTT	134.3 kb
371	02912-L02306	Exon 1	135-136	TGCTGCTTATCT-GGGAAGGCGATG	41204 kb to ACSL4 gene
		start codon	*295-297 (ex 2)*		

Mutations/deletions in the long OPHN1 gene (400 kb) cause syndromic X-linked mental retardation with epilepsy, rostral ventricular enlargement and cerebellar hypoplasia.

The NM_002547.2 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2h. ACSL4 (=FACL4) gene, Xq22.3

Length (nt)	SALSA MLPA probe	ACSL4 Exon	Ligation site NM_022977.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2639-2641 (ex 17)*		
190 ~	02155-L15826	Exon 17	2534-2535	TAAGCCCAGAGC-CATGGACCCCTG	24.0 kb
148	02935-L02326	Exon 12	1880-1881	ATGTCTGCTTCT-GCTGCCCAATTG	65.1 kb
248	03512-L23557	Exon 1	173-174	GTCCCAGCGCTA-GCGGGCACGCGG	1390 kb to PAK3 gene
		start codon	*506-508 (ex 4)*		

~ The 190 nt probe has been marked as more variable by some users. From version B1 onwards the hybridising sequence of this probe has been elongated. Please notify us on variable results obtained with our probes.

The ACSL4 (=FACL4) gene, total length of 92 kb, is not identified as an MRX gene. It encodes a form of Long chain acyl-CoA synthetase (LACS) and is expressed in several tissues, including brain. It has been suggested that the absence of ACSL4 might play a role in the development of mental retardation or other signs associated with Alport syndrome in these patients. Please note that the ACSL4 gene is in a region that is duplicated in some healthy individuals according to the http://dgv.tcag.ca/dgv/app/home database.

The NM_022977.2 sequence represents transcript variant 2 and is a reference standard in the NCBI RefSeqGene project.

Table 2i. PAK3 gene, Xq22.3

Length (nt)	SALSA MLPA probe	PAK3 Exon	Ligation site NM_002578.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*627-629(ex 5)*		
385	02908-L03178	Exon 5	704-705	CGGGATTCTTCA-GCACTCAACCAC	40.5 kb
418	02909-L02303	Exon 10	1301-1302	CCACCCTCTGCT-GAAAATGCCAAT	52.8 kb
400	03521-L02304	Exon 17	2063-2064	ACTAATGGAACT-CCAGAGCTCCAG	4.0 kb
481	02911-L02305	Exon 18	2234-2233 reverse	TTAATTGCTTCC-TTTGCAGCGATA	113 kb to DCX gene
		stop codon	*2259-2261 (ex 18)*		

Mutations in the PAK3 gene have been reported as being the cause of non-syndromic mental retardation (MRX30). The total length of the PAK3 gene is 1370 kb.

The NM_002578.3 sequence represents transcript variant 2 and is a reference standard in the NCBI RefSeqGene project.

Table 2j. DCX gene, Xq23

Length (nt)	SALSA MLPA probe	DCX Exon	Ligation site NM_178152.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*1213-1215 (ex 7)*		
275	04124-L03481	Exon 4 (5)	864-865	GATGATGTGTTT-ATTGCCTGTGGT	67.9 kb
215	04123-L15828	Exon 3 (4)	756-757	GTCCTCACTGAT-ATCACAGAAGCC	9.8 kb
319 *	04121-L08390	Exon 2	NM_000555.3 295-296*	CAGGCTATGGAT-TCATTTACAACT	4675 kb to AGTR2 gene
		start codon	*118-120 (ex 3)*		

Mutations in the DCX gene (118 kb) are found to result in Lissencephaly ('smooth brain'), characterized by mental retardation and seizures.

- P061 Lissencephaly: contains more probes for DCX.

The NM_178152.2 sequence represents transcript variant 2 and is a reference standard in the NCBI RefSeqGene project.

* Probe 04121-L08390 targets a sequence that is not present in NM_178152.2.

Note: The DCX exon numbering has changed. From description version 17 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for the DCX gene. The 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 2j.

Table 2k. AGTR2 gene, Xq24

Length (nt)	SALSA MLPA probe	AGTR2 Exon	Ligation site NM_000686.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*208-210 (ex 3)*		
355	02925-L02319	Exon 1	70-71	TGAGAGAACGAG-TAAGCACAGAAT	2.1 kb
130	13917-L02320	Exon 3	812-813	TTTCCCACCTGA-GAAATATGCCCA	20367 kb to ARHGEF6
		stop codon	*1297-1299 (ex 3)*		

Mutations in the AGTR2 gene have been reported as being the cause of non-syndromic mental retardation (MRX88), often accompanied by seizures. AGTR2 spans 4 kb of genomic DNA.
The NM_000686.4 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2l. ARHGEF6 gene, Xq26.3

Length (nt)	SALSA MLPA probe	ARHGEF6 Exon	Ligation site NM_004840.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2791-2793 (ex 22)*		
202	02902-L04460	Exon 19	2439-2440	GATGCTCAAATC-CTTAAAGTGATC	31.9 kb
172	16857-L19651	Exon 9	1414-1415	ACAAAGTAGGAG-GTTGTCTACTGA	38.3 kb
443	16856-L19650	Exon 4	829-830	GTGGACGTTCCT-CTTCTCTTAGTG	35.5 kb
346	03719-L02293	Exon 1	532-533	CTAAAAAGACCA-TCTGTGATCCGG	11131 kb to FMR1 gene
		start codon	*463-465 (ex 1)*		

Mutations in the ARHGEF6 gene have been reported as being the cause of non-syndromic mental retardation (MRX46). ARHEF6 spans 115 kb of genomic DNA.
The NM_004840.2 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2m. FMR1 gene, Xq27.3

Length (nt)	SALSA MLPA probe	FMR1 Exon	Ligation site NM_002024.5	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*230-232 (ex 1)*		
166	02927-L03721	Exon 9	1059-1060	AAAAGCTAGAAG-CTTTCTCGAATT	12.9 kb
142	02928-L03720	Exon 16	1907-1908	ACTCCCGAACAG-ATAATCGTCCAC	556 kb to AFF2 gene
		stop codon	*2126-2128 (ex 17)*		

Defects in the FMR1 gene, spanning 40 kb, result in fragile X syndrome, characterised by moderate to severe mental retardation. Expansion of a trinucleotide repeat near the FMR1 gene is the most common defect of this gene. This expansion results in methylation of the promoter and inactivation of the FMR1 gene.

- ME029 FMR1/AFF2: The ME029 MS-MLPA probemix contains more FMR1 probes and allows detection of both copy number changes, as well as the detection of promoter methylation (in full mutation male samples) of the FMR1 and AFF2 (FMR2) genes. It is not possible to directly measure the length of the trinucleotide repeat by MLPA.

The NM_002024.5 sequence represents transcript variant ISO1 and is a reference standard in the NCBI RefSeqGene project.

Table 2n. AFF2 (FMR2) gene, Xq28

Length (nt)	SALSA MLPA probe	AFF2 Exon	Ligation site NM_002025.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*480-482 (ex 1)*		
154	03511-L04202	Exon 1	501-502	TCGACTTTTTCA-GAGACTGGGACT	161.1 kb
283	00493-L00066	Exon 3	978-979	GTCATAACCCTA-GCACTGTACTGG	175.5 kb
241	03516-L15823	Exon 5	1604-1605	CTCACTTCCATG-CATACTGCTGGA	118.4 kb
337	02932-L02323	Exon 11	2537-2538	GAACCAAGACCT-AACATCCCTTTG	31.3 kb
263	02933-L23673	Exon 20	4128-4129	CAGTGTCTCTCA-ACAACGTCTCCC	4734 kb to SLC6A8 gene
		stop codon	*4413-4415 (ex 21)*		

The long, 500 kb, AFF2 (FMR2) gene is located at close distance (550 kb) from FMR1 and spans almost 500 kb. Similar to FMR1, expansion of a trinucleotide near the promoter can result in inactivation of the gene. Inactivation of the AFF2 gene has been associated with mental retardation, premature ovarian failure and obsessive-compulsive disorder.

- ME029 FMR1/AFF2: contains more probes for AFF2. This ME029 probemix can also be used to detect AFF2 promoter methylation. No MLPA product is available to determine the length of the trinucleotide repeat in AFF2 exon 1.

The NM_002025.3 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Table 2o. SLC6A8 gene, Xq28

Length (nt)	SALSA MLPA probe	SLC6A8 Exon	Ligation site NM_005629.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*279-281 (ex 1)*		
208	01871-L15827	Exon 3 (4)	853-854	AGACTGTGCCAA-TGCCAGCCTGGC	2.5 kb
301	01876-L01445	Exon 8 (9)	1448-1449	ATCGCCTACCCG-CGGGCTGTCACG	706 kb to GDI1 gene
		stop codon	*2184-2186 (ex 13)*		

The SLC6A8 gene contains 13 exons and spans 9 kb. Mutations in SLC6A8 are reported to cause MRX.

- P049 SLC6A8: contains more probes for SLC6A8.

The NM_005629.3 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Note: The SLC6A8 exon numbering has changed. From description version 17 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for the SLC6A8 gene. The 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 2o.

Table 2p. GDI1 gene, Xq28

Length (nt)	SALSA MLPA probe	GDI1 Exon	Ligation site NM_001493.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*343-345 (ex 1)*		
136	16875-L19669	Exon 1	347-348	CCTGACCATGGA-CGAGGAATACGA	3.9 kb
378	16874-L23559	Exon 7	1117-1118	TGGATGACATCA-TCATGGAGAACG	
		stop codon	*1684-1686 (ex 11)*		

GDI1 is a small gene of 6 kb. However, the prevalence of GDI1 mutations (nonsense & missense) in nonspecific mental retardation may be 0.5 to 1%.

The NM_001493.2 sequence is a reference standard in the NCBI RefSeqGene project.

Note: Exon numbering might be different as compared to literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P106-C1 MRX sample picture

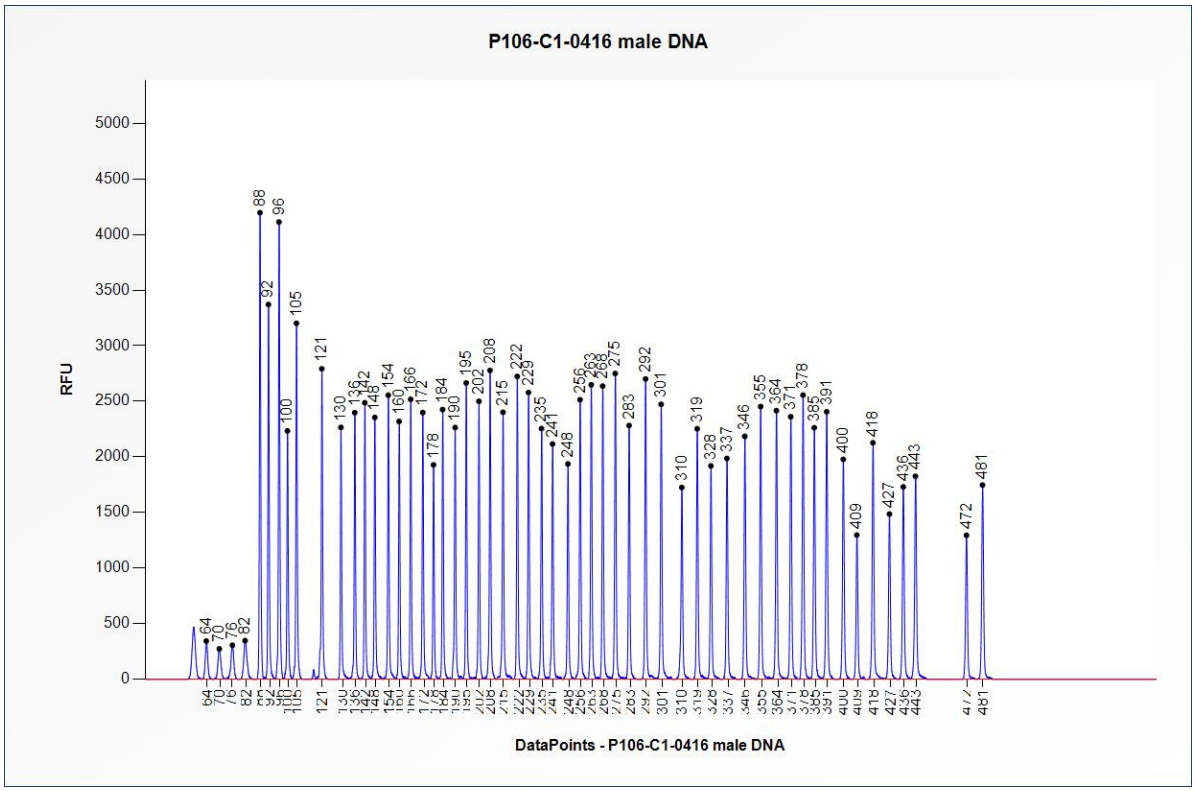

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P106-C1 MRX (lot C1-0416).

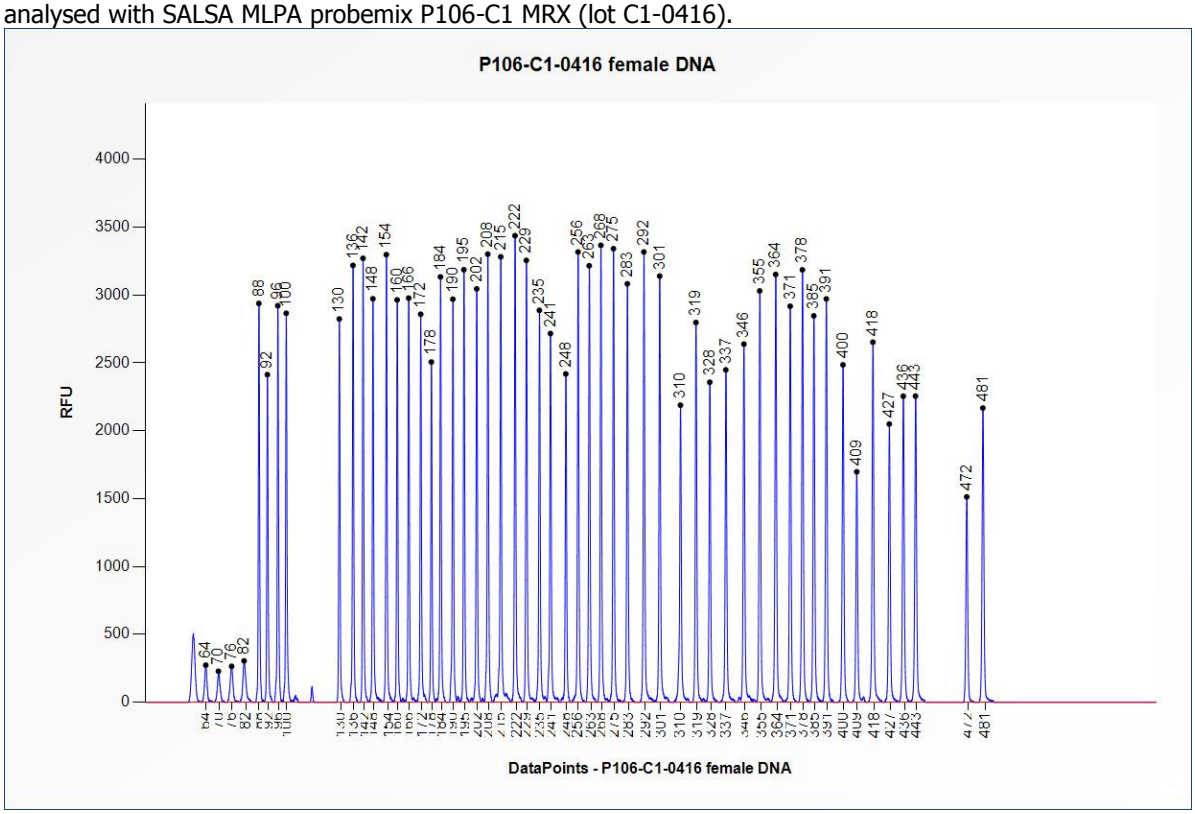

Figure 2. Capillary electrophoresis pattern of a sample of approximately 50 ng human female control DNA analysed with SALSA MLPA probemix P106-C1 MRX (lot C1-0416).

Implemented Changes – compared to the previous product description versions

Version 18 – 30 September 2016 (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).
- Several small textual changes throughout the document.

Version 17 – 29 February 2016 (55)
- DCX and SLC6A8 exon numbering adjusted in Table 2j and 2o, respectively.

Version 16 (53)
- Various textual and lay-out changes.
- Updated link for "Database of Genomic Variants".

Version 15 (49)
- 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).

Version 14 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 13 (46).
- Exon numbering of the DCX gene has been changed in Table 2j.
- Ligation sites of the probes targeting the DCX gene updated according to new version of the NM_reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Remark on transcript variant used and RefSeqGene standard added below Table 2.

Version 12 (46)
- New reference added on page 1.
- Warning added in Table 1, 229 nt probe 13669-L15822.
- Exon numbering of the DCX and AFF2 genes has been changed in Table 2.
- Data analysis method has been modified.
- Ligation sites of the probes targeting the FMR1 gene updated according to new version of the NM_reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various minor textual changes on page 1.
- Various minor layout changes.