

## SALSA MLPA KIT P106 MRX

Lot 0307, 1005, 0405

MENTAL RETARDATION (MR) is currently 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 2(9): 669-680). Among mentally retarded patients, an excess of males over females has long been noted and 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. Nineteen genes responsible for MRX have been identified so far.

This P106 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 MRX kit includes probes for 14 of the 19 MRX genes. For most genes, probes are present for only some of the exons. More kits for specific syndromes are available, see page...

This MLPA kit is designed to detect deletions/duplications of one or more exons of the MRX genes. Deletions of probe recognition sequences in males will be apparent by the absence of the probe amplification product. In female heterozygotes, a 35-50% reduced relative peak area of the amplification product of that probe is expected. However, mutations/polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Apparent deletions of a single exon therefore always require confirmation by other methods. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Please note that most defects in these genes are expected to be small (point) mutations, most of which will not be detected by this MLPA test. For FMR1 and FMR2, expansion of the trinucleotide repeat near the promotor is probably the most common cause of inactivation. This expansion can not be detected by this MLPA kit.

**SALSA® MLPA® kits are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. This kit is not CE/FDA certified for use in diagnostic procedures. SALSA MLPA kits are supplied with all necessary buffers and enzymes. Purchase of the SALSA MLPA test kits includes a limited license to use these products for research purposes.**

The use of this SALSA MLPA kit 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 kits

- P064 / P096 MR: Can be used to detect copy number changes of several autosomal genes / chromosomal regions that are implicated in mental retardation.
- P245 Microdeletion: Probes are included for 21 different microdeletion syndromes and can be used for primary screening of microdeletion syndromes.
- P036/P070 telomere: These probemixes contain one probe for every subtelomere.
- More kits for specific subtelomere analysis are available.
- For P106 syndrome-specific kits please see page 4-6.

### More information

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## MRX genes

- X-linked mental retardation associated with marXq28, or fragile X syndrome, is characterized by moderate to severe mental retardation, macroorchidism, large ears, prominent jaw, and high-pitched jocular speech. Expression is variable, with mental retardation being the most common feature. This phenotype is associated with mutations in the **FMR1 (FRAXA)** gene. Expansion of a trinucleotide repeats near the FMR1 gene is the most common defect of this gene. This expansion results in methylation of the promotor and inactivation of the FMR1 gene. A separate MLPA kit for FMR1 and FMR2 is in development and will allow detection of both copy number changes as well as detection of promotor methylation of these genes. It is not possible to directly measure the length of the trinucleotide repeat by MLPA. The FMR1 gene spans 40 Kb on chromosome Xq27.3.
- The **FMR2 (FRAXE)** gene is located at close distance (550 Kb) from FMR1 and spans almost 500 Kb. Similar to FMR1, expansion of a trinucleotide near the promotor can result in inactivation of the gene. Inactivation of the FMR2 gene has been associated with mental retardation, premature ovarian failure and obsessive-compulsive disorder.
- The **ARX** gene spans 11 Kb. Disruption / mutations in this gene causes severe X-linked infantile spasms and mental retardation. MRX is also caused by mutations of disruption due to translocation of the **ARHGEF6** gene or the **TM4SF2**. ARHGEF6 spans 115 Kb and TM4SF2 125 Kb.
- Some mutations in the **RPS6KA3** gene (110 Kb) cause mild mental retardation. Most mutations (incl. Truncating) cause Coffin-Lowry syndrome. Coffin-Lowry syndrome is characterized by mental retardation and strange fingers, large ears etc.
- Mutations / deletions in the 400 Kb **OPHN1** gene cause syndromic X-linked mental retardation with epilepsy, rostral ventricular enlargement and cerebellar hypoplasia.
- **GDI1** is a small gene of 6 Kb. The prevalence of GDI1 mutations (nonsense & missense) in nonspecific mental retardation may be 0.5 to 1%.
- A variable phenotype of mental retardation X-linked is caused by mutation in the gene encoding the polyglutamine-binding protein-1 (**PQBP1**). Changes in an AG6 repeat in the fourth coding exon of the PQBP1 gene causes mental retardation. This gene is 5 Kb long.
- The **SLC6A8** gene contains 13 exons, spans about 8.5 kb of genomic DNA, and is approximately 36 kb centromeric of ALD. Exon 1 occurs within a CpG island, and the gene is transcribed towards the telomeric end. Mutations in SLC6A8 are reported to cause MRX.
- The **FACL4 (=ACSL4)** gene is not identified as a 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 FACL4 might play a role in the development of mental retardation or other signs associated with Alport syndrome. In these patients mental retardation or other signs associated with Alport syndrome in these patients. Mutations in an other non-MRX gene include in this probemix, **DCX**, is found to implicate Lissencephaly ('smooth brain'), which is characterised with mental retardation and seizures.

## Data analysis

The P106 MRX probemix contains 44 different probes with amplification products between 130 and 463 nt. In addition, it contains 7 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, one ligation-dependent control fragment at 92 nt, and two Y-fragments at 106 nt and 118 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 normalized with a more robust method. The signals of all probes should be intra-normalized against every single probe separately, thereby creating as many ratios as there are probes. The median of all produced ratios gives an estimate of the final probe ratio, or ploidy status, of the sample's probes sequences in an MLPA mix. This way, the signal of each probe will be used as a normalization constant (population normalization). With the normalization constant, the ratio of each probe between reference and patient sample is determined. When only small numbers of samples are tested, visual comparison of peak profiles should be sufficient to easily identify copy number changes. Comparison of results should preferably be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most deletions can be done by FISH.

This probemix was developed by J. Coffa & J.P. Schouten at MRC-Holland. In case the results obtained with this probemix lead to a scientific publication, it would be very much appreciated if the first probemix designer could be made a coauthor. Info/remarks/suggestions for improvement: [info@mlpa.com](mailto:info@mlpa.com).

## SALSA MLPA P106 MRX probemix

Length (nt)	SALSA MLPA probe	Gene	Chromosomal position
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
92	Ligation dependent control fragment at 2q14		
106	Y-fragment: Specific for the Y chromosome for UTY at Y-014.5		
118	Y-fragment: Specific for the Y chromosome for DBY (DDX3Y) at Y-014.0		
130	1690-L0423	Reference probe	Xq11.2
136	2916-L4199	GDI1	Xq28
142	2928-L3720	FMR1	Xq27.3
148	2935-L2326	FACL4	Xq22.3
154	3511-L4202	FMR2	Xq28
160	2903-L2297	TM4SF2	Xp11.4
166	2927-L3721	FMR1	Xq27.3
172	2901-L2295	ARHGEF6	Xq26
178	2907-L2301	RPS6KA3	Xp22.2
184	2155-L1607	FACL4	Xq22.3
193	3514-L2291	ARX	Xp22.1
202	2902-L4460	ARHGEF6	Xq26
211	4123-L3480	DCX	Xq23
220	2898-L4200	ARX	Xp22.1
229	2922-L4201	IL1RAPL1	Xp22.1
238	3516-L2322	FMR2	Xq28
247	3512-L1606	FACL4	Xq22.3
256	2933-L2324	FMR2	Xq28
265*	2904-L2298	TM4SF2	Xp11.4
274	4124-L3481	DCX	Xq23
283	0493-L0369	FMR2	Xq28
292	2920-L2314	IL1RAPL1	Xp22.1
301	1876-L1445	SLC6A8	Xq28.1
310	2918-L2878	PQBP1	Xp11.23
319	4121-L3478	DCX	Xq23
328	2921-L2315	IL1RAPL1	Xp22.1
337	2932-L2323	FMR2	Xq28
346	3719-L2293	ARHGEF6	Xq26
355	2925-L2319	Agtr2	Xq24
364	2906-L2300	RPS6KA3	Xp22.2
373	2912-L2306	OPHN1	Xq12
378	2917-L2311	GDI1	Xq28
385	2908-L3178	PAK3	Xq23
391	3520-L2313	PQBP1	Xp11.23
400	3521-L2304	PAK3	Xq23
409	2913-L2307	OPHN1	Xq12
418	2909-L2303	PAK3	Xq23
427	2923-L2317	IL1RAPL1	Xp22.1
436	2914-L2308	OPHN1	Xq12
445	3720-L2294	ARHGEF6	Xq26
454	3762-L2299	RPS6KA3	Xp22.2
463	1872-L1441	SLC6A8	Xq28.1
472	2915-L2309	OPHN1	Xq12
481	2911-L2305	PAK3	Xq23

\* This probe has been found to be duplicated in some healthy individuals.

**Note:** Exon numbering might be different as compared to literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: [info@mlpa.com](mailto:info@mlpa.com).

## P106 MRX probes arranged according to chromosomal location

### PQBP1 gene, 5 Kb

Length (nt)	SALSA MLPA probe	PQBP1 exon	Ligationsite NM_005710.1	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
		<i>Startcodon</i>	<i>258-259</i>		
310	2918-L2878	exon 1	158-159	ATGAGTACAT-GTTTACGGGA	4.0 Kb
391	3520-L2313	exon 4	570-571	AAGTTGGACC-GGAGCCATGA	

### TM4SF2 gene, 125 Kb

Length (nt)	SALSA MLPA probe	TM4SF2 exon	Ligationsite NM_004615.2	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
		<i>Startcodon</i>	<i>62-64</i>		
160	2903-L2297	exon 1	88-89	GGAGACCAAA-CCTGTGATAA	114 Kb
265	2904-L2298	exon 5	523-524	TGGTGTGCAG-AACTACACCA	

### ARX gene, 11 Kb

Length (nt)	SALSA MLPA probe	ARX exon	Ligationsite NM_139058.1	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
		<i>Startcodon</i>	<i>1-3</i>		
193	3514-L2291	exon 1	56-57	GCAAAAGTAA-ATCTCCAAC	9.5 Kb
220	2898-L4200	exon 4	1414-1413 rev.	CTGATGAAAG-CTGGGTGTCG	

### Related SALSA MLPA kit

- P189 RETT like: contains more probes for ARX

### IL1RAPL1 gene, 1370 Kb Distance from exon 6 to the end of the gene is > 285 Kb !

Length (nt)	SALSA MLPA probe	IL1RAPL1 exon	Ligationsite NM_014271.2	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
		<i>Startcodon</i>	<i>631-633</i>		
292	2920-L2314	exon 1	367-368	GCAAAACAATC-GGGCACTTTG	201,3 Kb
328	2921-L2315	exon 2	640-641	ATGAAAGCTC-CGATTCCACA	493,5 Kb
229	2922-L4201	exon 3	938-939	GCCCAAAGTG-CTGGACTCAG	384,9 Kb
427	2923-L2317	exon 6	1348-1349	CTGACTGATA-AGCCACCCAA	

### RPS6KA3 gene, 110 Kb

Length (nt)	SALSA MLPA probe	RPS6KA3 exon	Ligationsite NM_004586.2	Partial sequence (20 nt adjacent to ligation site)	Distance to next probe
		<i>Startcodon</i>	<i>1-3</i>		
454	3762-L2299	exon 1	38-39	GGCAGAAGAT-GGCTGTGGAG	57 Kb
364	2906-L2300	exon 3	180-181	GGAAGGACAT-GAAAAGGCAG	53 Kb
178	2907-L2301	exon 21	2005-2006	CAGAGACTGA-CTGCTGCTCT	

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

OPHN1 gene, 400 Kb

Length (nt)	SALSA MLPA probe	OPHN1 exon	Ligationsite NM_002547.1	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>639-641</i>		
373	2912-L2306	exon 1	479-480	CTGCTTATCT-GGGAAGGCGA	134 Kb
409	2913-L2307	exon 4	833-834	GACGCTGCAG-TCATTTCAAGT	102 Kb
436	2914-L2308	exon 12	1695-1696	GCCCTTTCAG-AAGCTAACAG	133 Kb
472	2915-L2309	exon 20	2515-2516	TCACCAGCAG-CATAGAACCC	

FACL4 (=ACSL4) gene

Length (nt)	SALSA MLPA probe	FACL4 exon	Ligationsite NM_022977.1	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>507-509</i>		
247	3512-L1606	promotor region	174-175	CCCAGCGCTA-GCGGGCACGC	65 Kb
148	2935-L2326	exon 10	1881-1882	GTCTGCTTCT-GCTGCCAAT	24 Kb
184	2155-L1607	exon 15	2535-2536	AGCCCAGAGC-CATGGACCCC	

DCX gene

Length (nt)	SALSA MLPA probe	DCX exon	Ligationsite NM_178151.1	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>132-133</i>		
319	4121-L3478	exon 1	295-296	GGCTATGGAT-TCATTTACAA	9.3 Kb
211	4123-L3480	exon 3	1054-1055	CCTCACTGAT-ATCACAGAAG	71.6 Kb
274	4124-L3481	exon 4	1133-1134	TGATGTGTTT-ATTGCTGTG	

Related SALSA MLPA kit

- P061 Lissencephaly: contains more probes for DCX

PAK3 gene, 1370 Kb

Length (nt)	SALSA MLPA probe	PAK3 exon	Ligationsite NM_002578.2	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>397-399</i>		
385	2908-L3178	exon 5	474-475	GGATTCTTCA-GCACTCAACC	41 Kb
418	2909-L2303	exon 10	1071-1072	ACCCTTGCT-GAAAATGCCA	32 Kb
400	3521-L2304	exon 17	1833-1834	TAATGGAAGT-CCAGAGCTCC	24 Kb
481	2911-L2305	exon 18	2004-2003 rev.	AATTGCTTCC-TTGACGCGA	

Agtr2 gene

Length (nt)	SALSA MLPA probe	IL1RAPL1 exon	Ligationsite NM_000686.3	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>463-466</i>		
355	2925-L2319	exon 1	25-26	AGAGAACGAG-TAAGCACAGA	

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

### ARHGEF6 gene, 115 Kb

Length (nt)	SALSA MLPA probe	ARHGEF6 exon	Ligationsite NM_004840.2	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>1-3</i>		
346	3719-L2293	exon 1	522-523	AAAAAGACCA-TCTGTGATCC	344 Kb
445	3720-L2294	exon 4	829-830	GGACGTTCT-CTTCTCTTAG	379 Kb
172	2901-L2295	exon 9	1414-1415	AAAGTAGGAG-GTTGTCTACT	295 Kb
202	2902-L4460	exon 18	2439-2440	TGCTCAAATC-CTTAAAGTGA	

### FMR1 gene

Length (nt)	SALSA MLPA probe	FMR1 exon	Ligationsite NM_002024.3	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>220-223</i>		
166	2927-L3721	9	1049-1050	AAGCTAGAAG-CTTTCTCGAA	74 Kb
142	2928-L3720	16	1897-1898	TCCCGAACAG-ATAATCGTCC	

More FMR1 probes will be available in a separate FMR1 / FMR2 probemix.

### FMR2 gene

Length (nt)	SALSA MLPA probe	FMR2 exon	Ligationsite NM_002025.1	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>480-483</i>		
154	3511-L4202	exon 2	501-502	GACTTTTTCA-GAGACTGGGA	10.2 Kb
283	0493-L0369	exon 3	978-979	CATAACCCTA-GCACTGTACT	175.5 Kb
238	3516-L2322	exon 5	1604-1605	CACTTCCATG-CATACTGCTG	121.0 Kb
337	2932-L2323	exon 11	2537-2538	ACCAAGACCT-AACATCCCTT	28.7 Kb
256	2933-L2324	exon 20	4128-4129	GTGTCTCTCA-ACAACGTCTC	

More FMR2 probes will be available in a separate FMR1 / FMR2 probemix.

### GDI1 gene, 6 Kb

Length (nt)	SALSA MLPA probe	GDI1 exon	Ligationsite NM_001493.1	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>81-83</i>		
136	2916-L4199	1	85-86	TGACCATGGA-CGAGGAATAC	4 Kb
378	2917-L2311	5	855-856	GATGACATCA-TCATGGAGAA	

### SLC6A8 gene

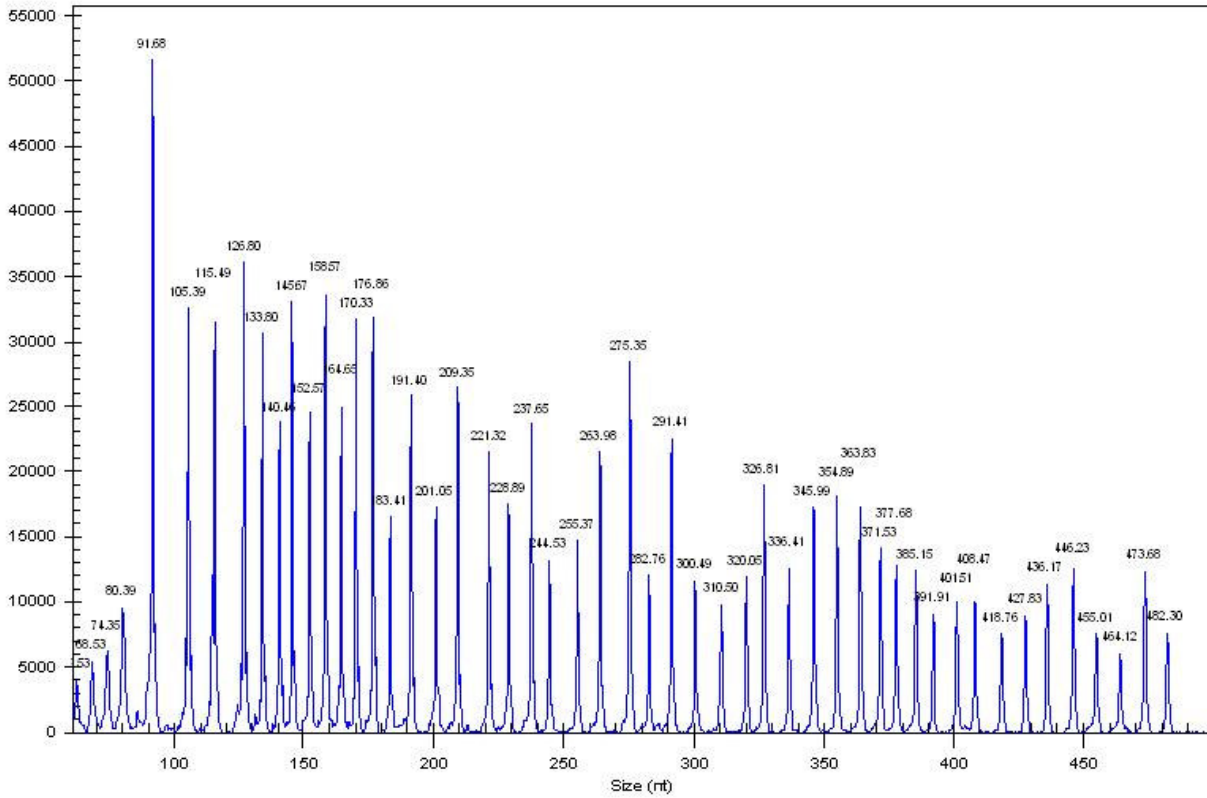
Length (nt)	SALSA MLPA probe	SLC6A8 exon	Ligationsite NM_005629.1	Sequence at Ligation site	Distance to next probe
		<i>Startcodon</i>	<i>639-640</i>		
463	1872-L1441	4	1391-1392	CTGTGTCTGG-AAGGGGGTCA	1.5 Kb
301	1876-L1445	8	1808-1809	CGCTACCCG-CGGGCTGTCA	

### Related SALSA MLPA kit

- P049 SLC6A8: contains more probes for SLC6A8.

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## SALSA MLPA kit P106 MRX sample picture



**Figure 1.** Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P106 MRX (lot 0307).