

SALSA MLPA KIT P015-D2 MECP2

Lot 0708. This lot has many changes as compared to previous lot. Several new MECP2 probes and probes centromeric and telomeric of MECP2 are included. In addition, some probes for the CDKL5, ARX and NTNG1 genes, involved in RETT-like syndrome, are included.

MECP2 is located at Xq28, close to the small pseudoautosomal region 2 (PAR2), at the q-telomere of chromosome X. Disruption of MECP2 in males is usually lethal. Disruption in females can cause RETT syndrome, one of the most common causes of mental retardation in females, with an incidence of 1 in 10.000-15.000. RETT syndrome is probably caused by inactivation of the single normal MECP2 copy by DNA-methylation. Female RETT syndrome patients are mosaic for MECP2-expression because X-chromosome inactivation is random.

Duplication of the complete MECP2 gene was identified as the cause of severe mental retardation with progressive spasticity in some males (Van Esch H. et al 2005 *Am. J. Hum. Genet.* 77, 442-453). MECP2 mutations have also been found in patients with a clinical diagnosis of Angelman syndrome. The most common cause of Angelman syndrome can be detected with SALSA MLPA kit ME028 Prader Willi / Angelman.

The MECP2 gene contains 4 exons. Start codon is close to the end of exon 2 and stop codon is in exon 4. The 3'UTR is unusually long: ~8.5 Kb.

This P015-D2 MECP2 probemix contains probes for each of the four exons. In addition, it contains several probes for genes in close proximity with MECP2. Some of these probes are located within the pseudoautosomal region 2 (PAR2). Although close to the small pseudoautosomal region PAR2, MECP2 is not pseudoautosomal and is not present on the Y-chromosome.

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the MECP2, CDKL5 and ARX genes that are located on the X-chromosome, and the autosomal NTNG1 gene. Deletions of X chromosome 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 a probe is expected. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation by other methods. 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.

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

- P189 CDKL5: RETT like syndrome (CDKL5, NTNG1, ARX genes)
- ME028 Prader Willi / Angelman syndrome: For both copy numbers as methylation detection of Prader Willi / Angelman region.

More information

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References of SALSA MLPA kit P015 MECP2

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- Zahorakova D et al. (2007). Mutation analysis of the MECP2 gene in patients of Slavic origin with Rett syndrome: novel mutations and polymorphisms. *J Hum Genet.* 2007;52(4):342-8. Epub 2007 Feb 15.
- Friez, MJ et al. (2006) Recurrent Infections, Hypotonia, and Mental Retardation Caused by Duplication of MECP2 and Adjacent Region in Xq28. *Pediatrics* 2006 Nov 6.
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- Ravn K, et al. (2005). Large genomic rearrangements in MECP2. *Hum Mutat;* 25(3):324.
- Erlandson A et al. (2003). Multiplex ligation-dependent probe amplification (MLPA) detects large deletions in the MECP2 gene of Swedish Rett syndrome patients. *Genet Test.;* 7(4):329-32.

Data analysis

The P015-D2 MECP2 probemix contains 43 MLPA probes with amplification products between 130 and 463 nt. In addition, it contains 10 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 two Y-fragments at 105 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 can be intra-normalized by dividing the peak area of each amplification product by the total area of only the reference probes in the probemix (block normalization). Secondly, 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 that no changes occurred in the genomic regions targeted by the reference probes. It is strongly recommended using reference and patient samples of the same sex to minimize variation, as intersex comparison makes analysis more difficult. Sex determination can also be done by visual examination of the electropherogram.

When only small numbers of samples are tested, visual comparison of peak profiles should be sufficient to 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 exons deletions and amplifications can be done by e.g. Southern blots or long range PCR. Confirmation of most large deletions can be done by FISH.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

This probemix was developed by R Vijzelaar and 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.

SALSA MLPA P015-D2 MECP2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position				
		reference	MECP2	CDKL5	ARX	NTNG1
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					
118*	Y-fragment: Specific for the Y chromosome					
130*	Reference probe 05169-L04550	8q24				
136*	GDI1 probe 02916-L04199		Xq28			
142*	CDKL5 probe 06466-L06567			Exon 10		
148*	NTNG1 probe 06478-L06568				1p13 Exon 1	
154*	ARX probe 06453-L06569			Exon 2		
160*	Reference probe 08222-L07936	10q26				
166*	SLC6A8 probe 01879-L01448		Xq28			
172*	IRAK1 probe 10835-L12500		Xq28			
178*	MECP2 probe 10836-L11493			Exon 1		
184*	CDKL5 probe 06458-L05984			Exon 3		
190*	MECP2 probe 10839-L11496			Exon 3		
196*	Reference probe 08747-L11148	10q21				
202*	MECP2 probe 03409-L02797			Exon 1		
208*	MECP2 probe 10842-L12494			Exon 4		
214*	MECP2 probe 03770-L13387			Exon 1		
220*	FVIII probe 06288-L05892		Xq28			
232*	ARX probe 06455-L05981			Exon 5		
238*	L1CAM probe 07051-L06660		Xq28			
247*	MECP2 probe 03768-L03229			Exon 2		
256	MECP2 probe 01769-L01333			Exon 4		
265*	Reference probe 01306-L11227	5q35				
274	MECP2 probe 01768-L01332			Exon 4		
283*	CDKL5 probe 06462-L05988			Exon 6		
292	MECP2 probe 02002-L01335			Exon 1		
301*	NTNG1 probe 06487-L06013				1p13 Exon 5	
310*	Reference probe 01789-L01353	13q14				
319*	DKC1 probe 08629-L08645		Xq28			
328*	CDKL5 probe 06471-L05997			Exon 15		
338	MECP2 probe 01347-L12498			Exon 4		
346*	MECP2 probe 10841-L11498			Exon 4		
356	MECP2 probe 01348-L12499			Exon 3		
362*	Reference probe 03013-L02453	9q34				
372	MECP2 probe 01349-L12497			Exon 2		
382*	IDH3G probe 01887-L01456		Xq28			
391*	MECP2 probe 10840-L11497			Exon 3		
400*	CDKL5 probe 06465-L05991			Exon 9		
409*	Reference probe 01796-L01359	13q14				
418	IRAK1 probe 01770-L01334		Xq28			
427*	NTNG1 probe 06488-L06014				1p13 Exon 6	
436*	NTNG1 probe 06483-L06009				1p13 Exon 3	
445*	FLNA probe 04138-L03495		Xq28			
454	VAMP7 (SYBL1) probe 01094-L00659		Xq28- PAR			
463*	Reference probe 02674-L02141	11q23				

* New in version D2 (from lot 0708 onwards)

Note: Please notify us of any mistakes. The identity of the genes detected by the reference probes and the complete sequences detected by all probes are available on request: info@mlpa.com.

P015-D2 probes arranged according to chromosomal location

MECP2 gene / Xq28 region (from telomere to centromere)

Length (nt)	SALSA MLPA probe	Gene / exon	Ligation site NM_004992.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		<i>Q-telomere</i>			<i>90.0 Kb</i>
454	01094-L00659	VAMP7 gene (PAR region)		TGTGGGAAAAGT-GTTTCCATTCTG	957.0 Kb
220*	06288-L05892	FVIII gene		TTCAGGGAGTCT-GGCCAAGGAAAA	218.1 Kb
319*	08629-L08645	DKC1 gene		CATGATGTGCTT-GATGCTCAGTGG	331.9 Kb
136*	02916-L04199	GDI1 gene		CCTGACCATGGA-CGAGGAATACGA	79.7 Kb
445*	04138-L03495	FLNA gene		TGACGGCACGTA-TACAGTGGCCTA	221.7 Kb
		<i>start codon</i>	<i>227-229</i>		
214*	03770-L13387	MECP2 exon 1	1093 nt before exon 1	GCAAGAATGTTA-GTTTGCTGTCTG	0.7 Kb
292	02002-L01335	MECP2 exon 1	381 nt before exon 1, reverse	GGGACGCCTGTT-TGCGCTGCTCTG	0.1 Kb
202*	03409-L02797	MECP2 exon 1	318 nt before exon 1	CATTAATCCTTA-ACATTCAAATTC	0.4 Kb
178*	10836-L11493	MECP2 exon 1	52-53	GGGCTGTGGTAA-AAGCCGTCCGGA	5.3 Kb
247*	03768-L03229	MECP2 exon 2	44 nt before exon 2	GAAAAAGGTCGT-GCAGCTCAATGG	0.1 Kb
372	01349-L12497	MECP2 exon 2	188-189	GACTCCCCAGAA-TACACCTTGCTT	59.6 Kb
190*	10839-L11496	MECP2 exon 3	47 nt before exon 3	ACTTGTTCTGCA-GACTGGCATGTT	0.1 Kb
391*	10840-L11497	MECP2 exon 3	214-215	GTCAGAAGACCA-GGACCTCCAGGG	0.1 Kb
356	01348-L12499	MECP2 exon 3	385-386	GCCCACCACTCT-GCTGAGCCCGCA	0.9 Kb
346*	10841-L11498	MECP2 exon 4	32 nt before exon 4	AGAGCCTCTAAT-TGTTCTTGTTGT	0.3 Kb
208*	10842-L12494	MECP2 exon 4	837-838	TCCTTGTC AAGA-TGCCTTTTCAAAA	0.3 Kb
338	01347-L12498	MECP2 exon 4	1243-1244	CTGAAGACCTGT-AAGAGCCCTGGG	0.4 Kb
274*	01768-L01332	MECP2 exon 4	1622-1623	TTTCATCCTCCA-TGCCAAGGCCAA	5.4 Kb
256	01769-L01333	MECP2 exon 4	7036-7037	CAGTAACACATA-GACTGTGCGCAT	5.8 Kb
		<i>stop codon</i>	<i>1685-1687</i>		
418	01770-L01334	IRAK1 exon 3		GCTGAGGATGGC-AACTTCGGGGG	0.2 Kb
172*	10835-L12500	IRAK1 exon 3		TTTATGAAGCTT-TTCCAGGCTCCC	154.4 Kb
238*	07051-L06660	L1CAM gene		CAGCGGGTGA AA-ACTACAGTGTCCG	70.3 Kb
382*	01887-L01456	IDH3G gene		TCCCCGAACTT-CGCACCCCGTCG	99.8 Kb
166*	01879-L01448	SLC6A8 gene		ACCCCGCTGGTC-TGCATGGTAAGG	

* New in version D2 (from lot 0708 onwards)

Note: Complete probe sequences are available on request: info@mlpa.com. Please notify us on any mistakes: info@mlpa.com.

CDKL5 gene

Length (nt)	SALSA MLPA probe	CDKL5 exon	Ligation site NM_003159.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	254-256		86 Kb from start gene to first probe
184	06458-L05984	exon 3	41 nt before exon 3	GAGCTTTGTAGT-TTGATGCGTGC	69.1 Kb
283	06462-L05988	exon 6	573-574	GCCAAATGGAGT-TCCACCTGAGAA	8.2 Kb
400	06465-L05991	exon 9	913-914	ATTGACCACTT-TTTACTATTCTAG	7.3 Kb
142	06466-L06567	exon 10	1026-1027	TCCTCAGTCCTT-GGAAAGAAGATA	17.8 Kb
328	06471-L05997	exon 15	2478-2479	TCTACCATCAGA-GAGCAGTTCTGG	40 Kb to end of gene
		<i>stop codon</i>	3344-3346		

More CDKL5 probes are present in SALSA MLPA kit P189 Rett-like. The CDKL5 probes are identical to the probes in the P189 probemix. The CDKL5 gene contains 21 exons. As the CDKL5 gene is very long, not all deletions / duplications will be detected by P015 MECP2 probemix. We therefore recommend to use SALSA MLPA kit P189 Rett-like additional to this P015 MECP2 probemix.

ARX gene

Length (nt)	SALSA MLPA probe	ARX exon	Ligation site NM_139058.1	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	1-3		3 Kb from start gene to first probe
154	06453-L06569	exon 2	1004-1005	CCGCACCACGTT-CACCAGCTACCA	8.4 Kb
232	06455-L05981	exon 5	1753-1754	CAGCACCCTCA-AGACCAAATGGA	
		<i>stop codon</i>	1687-1689		

More ARS probes are present in SALSA MLPA kit P189 Rett-like. The ARX probes are identical to the probes in the P189 probemix. The ARX gene contains 5 exons.

NTNG1 gene

Length (nt)	SALSA MLPA probe	NTNG1 exon	Ligation site NM_014917.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	603-605		
148	06478-L06568	exon 1	17 nt after exon 1	ATAAGGTTTGCT-ATCCTTCCACTT	184.6 Kb
436	06483-L06009	exon 3	1401-1402	GGATAAGGCTGT-TAAGACCAGCCG	83.1 Kb
301	06487-L06013	exon 5	227 nt after exon 5	TATGACTTTTCT-GACTACTCTTAA	72.7 Kb
427	06488-L06014	exon 6	1702-1703	GAATGTCTGCGA-CAACGAGCTCCT	
		<i>stop codon</i>			

More NTNG1 probes are present in SALSA MLPA kit P189 Rett-like. These four probes are identical to the in the P189 probemix. The NTNG1 gene contains 6 exons.

Note: Complete probe sequences are available on request: info@mlpa.com. Please notify us on any mistakes: info@mlpa.com.

SALSA MLPA kit P015-D2 MECP2 sample picture

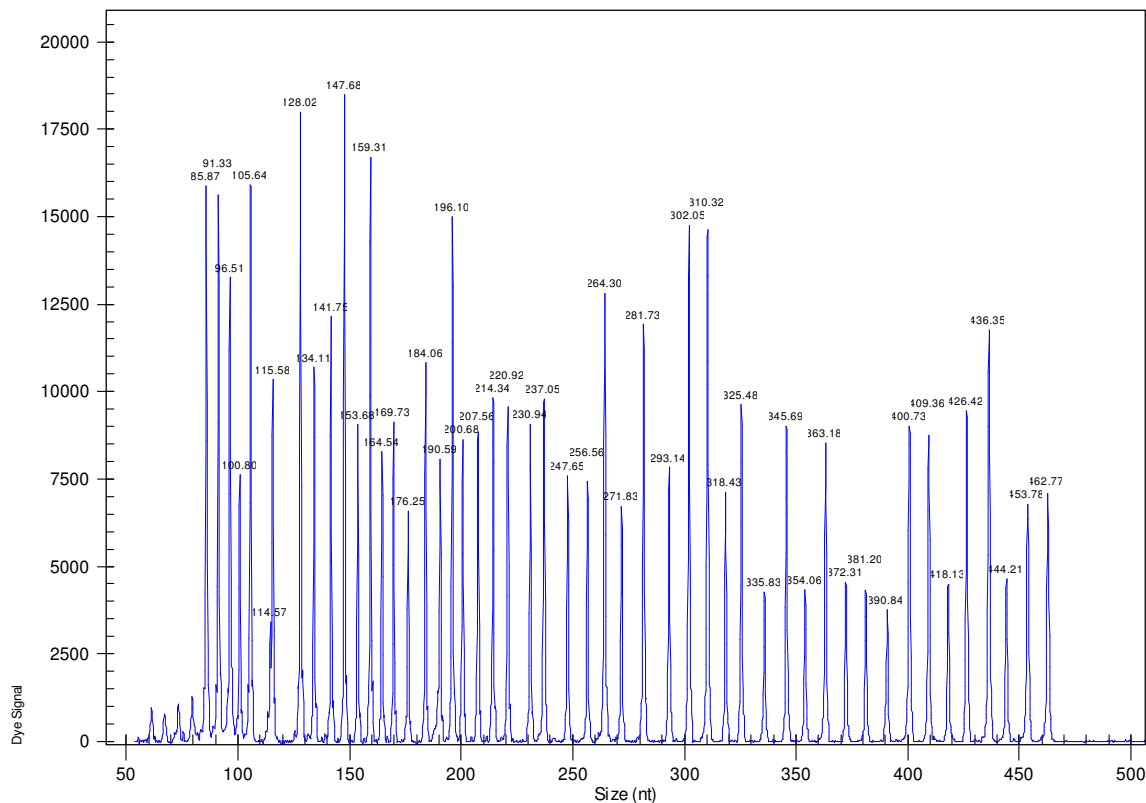


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P015-D2 MECP2 (lot 0708).

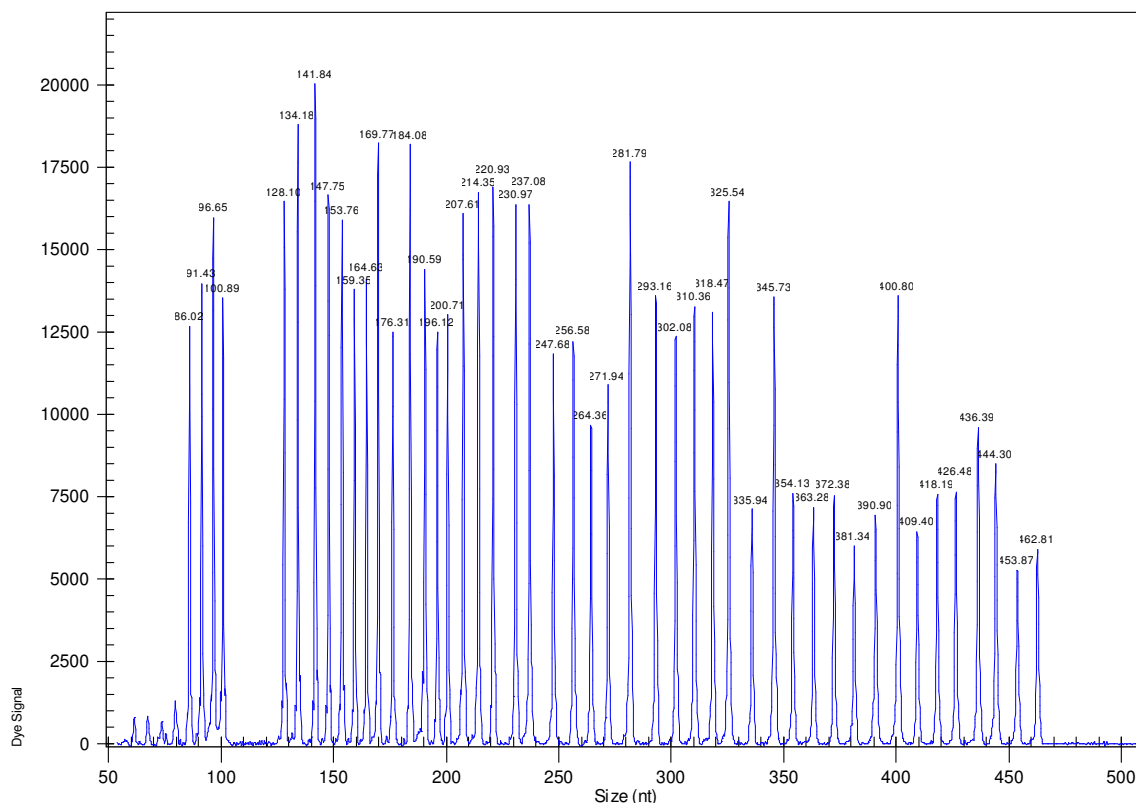


Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng human female control DNA analyzed with SALSA MLPA kit P015-D2 MECP2 (lot 0708).