

SALSA MLPA KIT P096 Mental Retardation-2

Lot 0307, 0406: Four WHSC1 probes have been added as compared to lot 0305

Copy number changes of several chromosomal regions are known to cause mental retardation. Examples are Smith-Magenis syndrome, Williams syndrome, 1p deletion syndrome and Miller-Dieker syndrome. These syndromes are not always easily diagnosed as the common clinical findings associated with a particular syndrome are often not present in each patient or are different between patients from different races. This SALSA MLPA P096 MLPA Mental Retardation-2 (MR2) kit provides a simple test to screen DNA samples for deletions and duplications of chromosomal regions that are involved in the following syndromes:

Wolf-Hirschhorn syndrome:	16 probes in the 4p telomeric region.
Cri du Chat syndrome:	6 probes in the 5p15 telomeric region.
Langer-Giedon syndrome:	6 probes in the 8q24 region implicated in Langer-Giedon syndrome.
WAGR syndrome:	8 probes in the 11p13-14 region implicated in WAGR syndrome.
Rubinstein-Taybi syndrome:	4 probes in the CREBB gene implicated in Rubinstein-Taybi disease.
Down syndrome:	2 probes for the chromosome 21 Downs syndrome critical region.
"Kabuki syndrome":	4 probes in the 8p region that was described to be involved in Kabuki syndrome. However, recent research does not confirm this.

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the MR2 genes. Heterozygote deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. However, mutations/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. 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.

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 MR1: Contains probes for 1p36, Williams, Smith-Magenis, Miller-Dieker, DiGeorge, Prader-Willi, Alagille, Saethre-Chotzen and Sotos syndrome.
- P106 MRX: X-linked 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.
- More probe for specific syndromes, e.g. RETT, DiGeorge, Prader Willi, Lissencephaly, Canavan and Williams syndrome, are available. Please see our website.
- For P096 syndrome-specific kits please see page 4-7.

More information

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Data analysis

The P096 MR2 probe mix 46 different probes with amplification products between 130 and 481 nt. In addition, it contains 5 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt and one ligation-dependent control fragment at 92 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

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 B. Beaumont & 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

SALSA MLPA P096 MR2 probemix

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Remarks	MapView 35
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA				
92	Synthetic control fragment at 2q14				
130	SPON2	3100-L3175	4p16.3	Wolf-Hirschhorn region	04-001.15
136	CTSB	1197-L0766	8p22	"Kabuki Syndrome"	08-011.8
142	CREBBP	3088-L3991	16p13.3	Rubinstein-Taybi Syndrome	16-003.8
148	TERT	3078-L2478	5p15.33	Cri du Chat Syndrome	05-001.32
154	FLJ20265	2005-L3588	4p16.3	Wolf-Hirschhorn region	04-000.5
160	FGFRL1	3099-L2502	4p16	Wolf-Hirschhorn region	04-001.0
166	PDCD6	1723-L3992	5p15.33	Cri du Chat Syndrome	05-000.35
172	CREBBP	3087-L2487	16p13.3	Rubinstein-Taybi Syndrome	16-003.8
178	CTBP1	3098-L2501	4p16.3	Wolf-Hirschhorn region	04-001.2
184	FDFT1	3083-L2483	8p23.1	"Kabuki Syndrome"	08-011.7
190*	WHSC1	6057-L6042	4p16.3	Wolf-Hirschhorn region	04-001.9
196**	HIPK3	0976-L6044	11p13	WAGR Syndrome	11-033.3
202	BDNF	3089-L2489	11p14	WAGR Syndrome	11-027.7
208*	WHSC1	6059-L6041	4p16.3	Wolf-Hirschhorn region	04-001.9
211**	DYRK1A	3791-L5919	21q22.2	Downs syndrome critical region	21-037.8
220	PAX6	3253-L2690	11p13	WAGR Syndrome	11-031.8
226*	WHSC1	6060-L5515	4p16.3	Wolf-Hirschhorn region	04-001.9
232**	LETM1	4190-L5920	4p16.3	Wolf-Hirschhorn region	04-001.8
238	SAMD12	0853-L1381	8q24	Langer-Giedion Syndrome	08-119.4
247	WT1	2755-L2204	11p13	WAGR Syndrome	11-032.4
256	FGFR3	2620-L4155	4p16.3	Wolf-Hirschhorn region	04-001.77
265	SEMA5A	3075-L2475	5p15.2	Cri du Chat Syndrome	05-009.3
274	GAK	1125-L0683	4p16	Wolf-Hirschhorn region	04-000.9
283	CRR9p	1126-L0684	5p15.33	Cri du Chat Syndrome	05-001.4
292	KCNJ6	3796-L3237	21q22.13	Downs syndrome critical region	21-038.1
301	CREBBP	3086-L2486	16p13.3	Rubinstein-Taybi Syndrome	16-003.8
310	FGFR3	3094-L2496	4p16.3	Wolf-Hirschhorn region	04-001.8
319	TRPS1	3082-L2482	8q24.12	Langer-Giedion Syndrome	08-116.6
328	CREBBP	3085-L3993	16p13.3	Rubinstein-Taybi Syndrome	16-003.8
337	TACC3	3096-L2499	4p16.3	Wolf- Hirschhorn region	04-001.7
346	EXT1	3080-L2480	8q24	Langer-Giedion Syndrome	08-119.0
355	PAX6	3092-L2492	11p13	WAGR Syndrome	11-031.8
364	MSRA	1202-L0787	8p23.1	"Kabuki Syndrome"	08-010.1
373	EXT1	3079-L3994	8q24	Langer-Giedion Syndrome	08-119.0
382	PAX6	3091-L2491	11p13	WAGR Syndrome	11-031.8
391	SLBP	3097-L2500	4p16.3	Wolf- Hirschhorn region	04-001.6
400	TRPS1	3081-L2481	8q24.12	Langer-Giedon Syndrome	08-116.6
409	CTNND2	3073-L2473	5p15.2	Cri du Chat Syndrome	05-011.5
418	WHSC1	3065-L2494	4p16.3	Wolf- Hirschhorn region	04-001.9
427	EIF3S3	1108-L0679	8q24.11	Langer-Giedon Syndrome	07-117.8
436	TERT	3761-L2477	5p15.33	Cri du Chat Syndrome	05-001.3
445*	WHSC1	6058-L5513	4p16.3	Wolf-Hirschhorn region	04-001.9
454	BDNF	3090-L3996	11p14	WAGR Syndrome	11-027.7
463	WHSC2	3058-L2495	4p16.3	Wolf- Hirschhorn region	04-002.0
472	WT1	2757-L2206	11p13	WAGR Syndrome	11-032.4
481	MFHAS1	1096-L0666	08p23.1	"Kabuki Syndrome"	08-008.8

* New in Lot 0406 + 0307

** Small change in length as compared to previous lots. No change in sequence detected.

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.

Wolf-Hirschhorn Region Probes

Wolf-Hirschhorn syndrome (WHS) (MIM 194190) is caused by partial deletion of the short arm of chromosome 4 and is characterized by severe growth retardation and mental defect, microcephaly, "Greek helmet" facies, and closure defects (cleft lip or palate, coloboma of the eye, and cardiac septal defects). Smaller deletions of the 4p subtelomeric region result in a milder phenotype. Most deletions are terminal and range in size from 1.9 to 3.5 Mb, although some interstitial deletions have been described. The minimum frequency of WHS is estimated at about 1 in 95.000. The WHS critical region is located at 4p16.3, approx. 2 Mb from the p-telomere.

The P096 probemix includes probes for the following genes located in the WHS chromosomal region:

- FLJ20265:** Hypothetical protein. Gene very close to telomere.
GAK: The gene for Cyclin G-Associated Kinase
FGFRL1: related to FGF receptors
SPON2: It has been recently proposed that Spon2 recognition of carbohydrate structures is essential for the activation of macrophages by pathogen-associated molecular patterns (PAMPs).
SLBP: Is the gene for subunit 2 of the replication factor C, which is involved in DNA replication.
TACC3: Close to translocation breakpoints in multiple myeloma, and is upregulated in various cancer cell lines
FGFR3: Defects of the FGFR3 gene are the cause of achondroplasia and other skeletal dysplasias.
LETM1: Seizures occurring in Wolf-Hirschhorn Syndrome patients were suggested to be caused by haplo-insufficiency for LETM1.
WHSC1: Good candidate to be responsible for many of the phenotypic features of WHS.
WHSC2: Wolf-Hirschhorn syndrome candidate-2.

WHS region probes arranged according to chromosomal location

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Distance to next probe	Distance from p-telomere
154	FLJ20265	2005-L3588	4p16.3	326 Kb	456 Kb
274	GAK	1125-L0683	4p16.3	228 Kb	782 Kb
160	FGFRL1	3099-L2502	4p16.3	88 Kb	1010 Kb
130	SPON2	3100-L3175	4p16.3	40 Kb	1098 Kb
178	CTBP1	3098-L2501	4p16.3	478 Kb	1138 Kb
391	SLBP	3097-L2500	4p16.3	20 Kb	1616 Kb
337	TACC3	3096-L2499	4p16.3	80 Kb	1636 Kb
310	FGFR3	3094-L2496	4p16.3	1 Kb	1716 Kb
256	FGFR3	2620-L4155	4p16.3	38 Kb	1717 Kb
229	LETM1	4190-L5920	4p16.3	53 Kb	1755 Kb
190	WHSC1	6057-L6042	4p16.3	6 Kb	1808 kb
418	WHSC1	3065-L2494	4p16.3	31 Kb	1814 Kb
445	WHSC1	6058-L5513	4p16.3	27 Kb	1845 kb
208	WHSC1	6059-L6041	4p16.3	21 Kb	1872 kb
226	WHSC1	6060-L5515	4p16.3	12 Kb	1893 kb
463	WHSC2	3058-L2495	4p16.3		1905 Kb

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.

Cri du Chat region Probes

The cause of the Cri du Chat (Cat cry) syndrome are deletions of the short arm of chromosome 5, that can vary considerably in size but usually include deletion of the TERT gene at 5p15.33 coding for the telomerase reverse transcriptase protein. Deletions are usually terminal but some interstitial deletions have been described.

Cri du Chat syndrome is characterised in young children by microcephaly, round face, hypertelorism, micrognathia, epicanthal folds, low-set ears, hypotonia, and severe psychomotor and mental retardation (OMIM 123450; <http://www.ncbi.nlm.nih.gov/Omim/>). One of the most characteristic features in newborn children is a high-pitched cat-like cry that is usually considered diagnostic for the syndrome.

The Cri du Chat syndrome appears to be one of the most common human deletion syndromes with an incidence varying between 1 in 20.000 to 1 in 50.000 births. The frequency in populations of profoundly retarded patients (IQ less than 20) is estimated to be approximately 1%.

Cri du Chat probes arranged according to chromosomal location

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Distance to next probe	Distance form p-telomere
166	PDCD6	1723-L3992	5p15.33	940 Kb	367 Kb
148	TERT, exon 15	3078-L2478	5p15.33	28 Kb	1307 Kb
436	TERT, exon 3	3761-L2477	5p15.33	63 Kb	1335 Kb
283	CRR9p	1126-L0684	5p15.33	8093 Kb	1398 Kb
265	SEMA5A	3075-L2475	5p15.2	2294 Kb	9490 Kb
409	CTNND2	3073-L2473	5p15.2		11785 Kb

"Kabuki" Syndrome probes

The cause of the Kabuki syndrome was reported to be interstitial deletions in the 8p region. A recent presentation at the 2004 American Society of Human Genetics meeting (Toronto, October 2004) however has placed doubts on the involvement of this 8p region in Kabuki syndrome. We included four probes in this region in this MR2 probemix. This region has been recently reported to have variable copy numbers in different individuals. These 8p probes might be replaced in future versions of this probemix.

Probes in the 8p region arranged according to chromosomal location

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Distance to next probe
481	MFHAS1	1096-L0666	8p23.1	1319 Kb
364	MSRA	1202-L0787	8p23.1	1600 Kb
184	FDFT1	3083-L2483	8p23.1	39 Kb
136	CTSB	1197-L0766	08p23.1	

"Downs Syndrome Critical Region" Probes

The cause of the Downs syndrome is trisomy for chromosome 21. Based on analysis of rare individuals with Downs syndrome phenotype but having an extra copy of only a part of chromosome 21, a Downs syndrome critical region has been proposed. Recent experiments with a mouse model have provided some doubts on the importance of this region. More SALSA MLPA probes in this region are available. Please enquire.

Downs syndrome region probes arranged according to chromosomal location

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Distance to next probe
211	DYRK1A	3791-L5919	21q22.2	492 Kb
292	KCNJ6	3796-L3237	21q22.13	

LANGER-GIEDION Syndrome probes

The cause of the Langer-Giedion syndrome (LGS) are the loss of functional copies of the TRPS1 and EXT1 genes on 8q24.11-8q24.13, usually due to interstitial deletions of this chromosomal region. Characteristics of LGS include multiple dysmorphic facial features including large, laterally protruding ears, a bulbous nose, an elongated upper lip, as well as sparse scalp hair, winged scapulae, multiple cartilaginous exostoses, redundant skin, and mental retardation. Please note however that mental retardation, associated with Langer-Giedion syndrome, can be mild (OMIM 150230; <http://www.ncbi.nlm.nih.gov/Omim/>).

Langer-Giedion region probes arranged according to chromosomal location

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Distance to next probe
319	TRPS1	3082-L2482	8q24	254 Kb
400	TRPS1	3081-L2481	8q24	896 Kb
427	EIF3S3	1108-L0679	8q24	1155 Kb
373	EXT1	3079-L3994	8q24	311 Kb
346	EXT1	3080-L2480	8q24	174 Kb
238	SAMD12	0853-L1381	8q24	

Related SALSA MLPA kit

- P215 EXT: contains many more probes for EXT1 and EXT2

WAGR Syndrome probes

The cause of the WAGR syndrome are deletions of the 11p13 chromosomal region at the WT1 gene. Characteristics of WAGR syndrome include Wilms tumor, aniridia, mental retardation and genitourinary abnormalities (OMIM 194072; <http://www.ncbi.nlm.nih.gov/Omim/>). Some patients combine WAGR syndrome with severe obesity.

WAGR syndrome region probes arranged according to chromosomal location

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Distance to next probe
454	BDNF	3090-L3996	11p14	41 Kb
202	BDNF	3089-L2489	11p14	4123 Kb
355	PAX6	3092-L2492	11p13	9 Kb
220	PAX6	3253-L2690	11p13	4 Kb
382	PAX6	3091-L2491	11p13	581 Kb
472	WT1	2757-L2206	11p13	46 Kb
247	WT1	2755-L2204	11p13	917 Kb
193	HIPK3	0976-L6044	11p13	

Related SALSA MLPA kits

- P118 WT1: contains many more probes for WT1
- P219 PAX6: contains more probes for PAX6

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.

RUBINSTEIN-TAYBI Syndrome probes

The cause of the Rubinstein-Taybi syndrome are defects in the gene encoding the transcriptional coactivator CREB-binding protein CREBBP (OMIM 180849; <http://www.ncbi.nlm.nih.gov/Omim/>). Most defects are due to small mutations. Most of these will not be detected by MLPA! Deletions of the 16p13.3 region including (part of) CREBBP might be the cause of the disease in 10-25% of the patients.

Frequency of Rubinstein-Taybi disease is estimated to be 1 per 300-500 in institutionalized patients with mental retardation over age 5 years.

CREBBP probes arranged according to chromosomal location

Length (nt)	Gene	SALSA MLPA probe	Chr. position	Distance to next probe	Partial sequence (20 nt adjacent to ligation site)
172	CREBBP	3087-L2487	Exon 2	30 Kb	CAGGTGAAAA-TGGCTGAGAA
328	CREBBP	3085-L3993	Exon 3	57 Kb	TGCTAACTTT-AACCAGACCC
301	CREBBP	3086-L2486	Exon 5	64 Kb	AAACGCAAAC-TGATACAGCA
142	CREBBP	3088-L3991	Exon 31		TTGTAGCATT-GTGAGAGCAT

Related SALSA MLPA kit

- P313 CREBBP: contains a probe for each of the 31 CREBBP exons.

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 kit P096 MR2 Sample picture

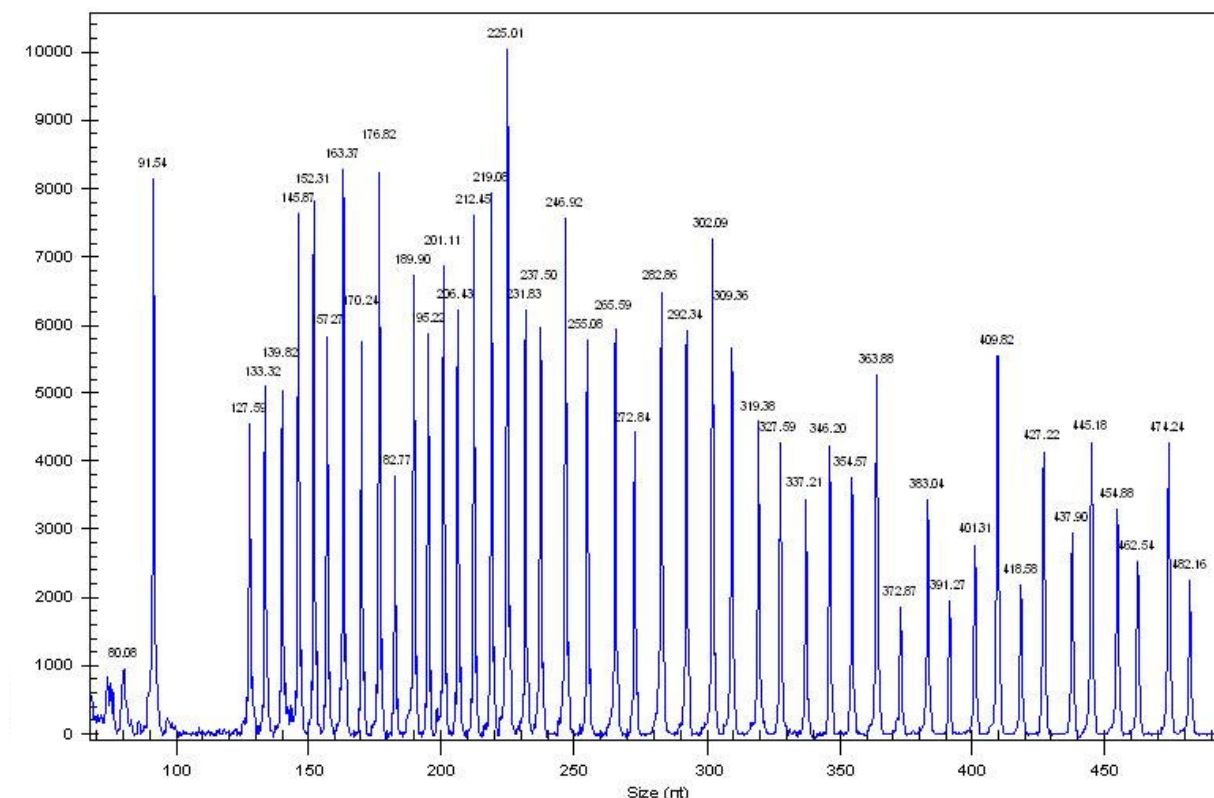


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P096 MR2 (lot 0307).