

SALSA MLPA KIT P245-A1 Microdeletion Syndromes

Lot 1207

This SALSA MLPA P245 Microdeletion Syndromes probemix has been developed to screen patients presenting with unexplained developmental delay and/or mental retardation for multiple microdeletion syndromes simultaneously. Results suggesting a deletion or duplication of a certain chromosomal region can be confirmed by other techniques or by a syndrome-specific MLPA kit. For nearly all syndromes included in this P245 probemix, there is a syndrome-specific SALSA MLPA probemix containing a larger number of probes targeted at the chromosomal region(s) involved (please see www.mlpa.com).

This P245 probemix has a limited number of probes for each specific chromosomal region and will therefore not detect all possible causes of the syndromes included. For example, 70% of Prader Willi cases are caused by a large deletion of a 15q region which should be detected by this P245 probemix. Methylation changes in the SNRPN gene region are another common cause of this syndrome but will not be detected by this P245 probemix. These methylation changes can be detected with the SALSA MS-MLPA kit ME028 Prader-Willi/Angelman. In case a particular phenotype of the patient suggests a specific microdeletion syndrome as a likely cause, we therefore recommend to us also a condition-specific MLPA probemix or another suitable technique to study the particular microdeletion syndrome suspected.

The microdeletion syndromes detected with this probemix P245 include:

1p36 deletion syndrome*	Cri du Chat syndrome, 5p15*	MECP2 / Xq28 duplication*
2p16 microdeletion	DiGeorge syndrome 22q11*	Rubinstein-Taybi syndrome
3q29 microdeletion	DiGeorge region 2, 10p15	Smith-Magenis syndrome*
9q22.3 microdeletion	Langer-Giedion syndrome, 8q	Sotos syndrome 5q35.3*
15q24 deletion syndrome*	Miller-Dieker syndrome, 17p*	Wagr syndrome
17q21 microdeletion*	NF1 microdeletion syndrome	Williams syndrome*
22q13 / Phelan-Mcdermid*	Prader-Willi / Angelman*	Wolf-Hirschhorn 4p16.3*

* Results obtained by Tommy Gerdes and colleagues in Copenhagen on patient samples are available on: <http://www.chromosomelab.dk/mlpa/examples-P245-vs2.html>

This SALSA MLPA kit is designed to detect deletions/duplications of several human chromosomal regions. For most regions, two or three probes are present. Included in this P245 probemix are two probes detecting a DNA sequence on chromosome Y and four probes detecting a chromosome X sequence. Deletions of these X and Y probe recognition sequences in males will result in the absence of the probe amplification product. In females, deletion of the X-chromosome sequences will result a 35-50% reduced relative peak area of the amplification product of that probe. For all other (autosomal) probes, deletions of probe recognition sequences will be apparent by a 35-50% reduced relative peak area of the amplification product. Mutations/polymorphisms very close to the probe ligation site may also result in a similar reduced relative peak area. Apparent deletions detected by a single probe always require confirmation by other methods.

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 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).

More information

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Related SALSA MLPA kits

- P064 MR-1/P096 MR-2: Contain probes for several microdeletion syndromes.
- P106 MRX: Probes for X-linked mental retardation.
- More probes for specific syndromes, e.g. RETT, DiGeorge, Prader Willi, Lissencephaly, Canavan and Williams's syndrome, are available. Please see pages 4 to 8.
- P036 Human telomere-3: Contains a probe for every human subtelomere.
- P070 Human telomere-5: Contains a probe for every human subtelomere. Can be used as confirmation kit of P036 Human telomere-3.
- P069 Human telomere-4: Same as P070 Human telomere-5, but does not contain probes for the acrocentric chromosome arms 13p, 14p, 15p, 21p and 22p.
- More kits for specific subtelomere analysis are available; see page 4.

Data analysis

This P245-A1 Microdeletion probemix contains 49 different MLPA probes with amplification products between 130 and 486 nt. In addition, 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, 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.

To create a more robust normalization, the signals of all probes are 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 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 exon deletions. 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 J.P. Schouten at MRC-Holland.

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

SALSA MLPA P245-A1 Microdeletion probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	Syndrome
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		
108	Y-fragment: Specific for the Y chromosome		
118	Y-fragment: Specific for the Y chromosome		
130	TNFRSF4 Probe 2269–L01761	1p36.33	1p36 deletion syndrome
136	GATA3 Probe 7632–L07317	10p	DiGeorge region 2 (10p)
142	PAFAH1B1 Probe 4120–L03532	17p13.3	Miller-Dieker region
148	MECP2 Probe 9310–L09999	Xq28	MECP2 / Xq28 duplication
154	NSD1 Probe 2595–L08077	5q35.3	Sotos syndrome
160	UBE3A Probe 4620–L00863	15q12	Prader-Willi / Angelman
166	GABRD Probe 4690–L07966	1p36.33	1p36 deletion syndrome
172	CREBBP Probe 3087–L02487	16p13.3	Rubinstein-Taybi syndrome
178	GNB1 Probe 2890–L07968	1p36.33	1p36 deletion syndrome
184	MECP2 Probe 9311–L10002	Xq28	MECP2 / Xq28 duplication
190	SEMA7A Probe 8380–L10003	15q24.1	"15q24 deletion syndrome"
196	CLDN5 Probe 1218–L06270	22q11.21	DiGeorge syndrome
202	MECP2 Probe 3409–L02797	Xq28	MECP2 / Xq28 duplication
208	GP1BB Probe 5464–L10114	22q11.21	DiGeorge syndrome
214	NDN Probe 6282–L01542	15q11.2	Prader-Willi / Angelman
220	PAX6 Probe 3253–L02690	11p13	Wagr syndrome
226	MAPT Probe 7856–L08385	17q21.31	"17q21 microdeletion"
232	LETM1 Probe 4190–L05920	4p16.3	Wolf-Hirschhorn region
238	PAFAH1B1 Probe 1443–L08394	17p13.3	Miller-Dieker region
247	SNRPN Probe 2026–L10004	15q12	Prader-Willi / Angelman
253*†	SHANK3 Probe 10181–L11409	22q13.33	22q13 / Phelan-Mcdermid
260‡	NF1 Probe 3778–L11180	17q11.2	NF1 microdeletion syndrome
267‡	FANCL Probe 8386–L11411	2p16.1	"2p16.1 deletion syndrome"
274	LRR48 Probe 1452–L00936	17p11.2	Smith-Magenis syndrome
283	CRR9 Probe 1126–L00684	5p15.3	Cri du Chat syndrome
292	SNRPN Probe 1318–L07970	15q12	Prader-Willi / Angelman
297	DMD Probe 1412–L01059	Xp21.2	Chromosome X control probe
303	LLGL1 Probe 1453–L08499	17p11.2	Smith-Magenis syndrome
310	ELN Probe 1333–L00876	7q11.23	Williams syndrome
319	TGFBR1 Probe 4652–L04036	9q22.33	"9q22.3 deletion syndrome"
325	CYP1A1 Probe 6811–L06406	15q24.1	"15q24 deletion syndrome"
335	NF1 Probe 2508–L02620	17q11.2	NF1 microdeletion syndrome
342	MAPT Probe 7857–L08501	17q21.31	"17q21 microdeletion"
349	Hs.538604 Probe 1232–L07388	10p15.1	DiGeorge region 2 (10p)
359	DLG1 Probe 8395–L08249	3q29	"3q29 deletion syndrome"
364	ELN Probe 1336–L00878	7q11.23	Williams syndrome
373	SNAP29 Probe 1235–L00773	22q11.21	DiGeorge syndrome
382*	SHANK3 Probe 10182–L11174	22q13.33	22q13 / Phelan-Mcdermid
391	LIMK1 Probe 1337–L02333	7q11.23	Williams syndrome
401	TRPS1 Probe 3081–L07411	8q24.12	Langer-Giedion syndrome
409	TGFBR1 Probe 4653–L10006	9q22.33	"9q22.3 deletion syndrome"
418	DLG1 Probe 8401–L08255	3q29	"3q29 deletion syndrome"
427	EIF3S3 Probe 1108–L00679	8q24.11	Langer-Giedion syndrome
436	TERT Probe 3761–L02477	5p15.33	Cri du Chat syndrome
445	WHSC1 Probe 6058–L05513	4p16.3	Wolf-Hirschhorn region
454	NSD1 Probe 2600–L02071	5q35.3	Sotos syndrome
465‡	RAI1 Probe 9440–L11412	17p11.2	Smith-Magenis syndrome
472	CRHR1 Probe 7859–L07620	17q21.31	"17q21 microdeletion"
486*	REL Probe 9860–L10628	2p16.1	"2p16.1 deletion syndrome"

*New from lot 1107 onwards. ‡ Change in length from lot 1107 onwards as compared to previous lots. † The 253 nt SHANK3 probe has been reported to be variable by some users and will be replaced in a future lot.

P245-A1 Microdeletion probes arranged to chromosomal location

1p36 deletion syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	2270-L01762	TNFRSF18	P069 / P070 probe for 1p36		8 Kb
130	2269-L01761	TNFRSF4	01-001.14	GCCGGCCAGCAA-TAGCTCGGACGC	16 Kb
	2271-L01763	CAB45	P036B probe for 1p36		54 Kb
	6778-L04070	SCNN1D	P036D probe for 1p36		539 Kb
178	2890-L07968	GNB1	01-001.75	CTAAGATCGGAA-GATGAGTGAGCT	200 Kb
166	4690-L07966	GABRD	01-001.95	CGGCGACTACGT-GGGCTCCAACCT	

- Many more 1p36 probes are present in the P147 probemix.
- Deletions / duplications in the 1p36 region have been reported to be a frequent cause of MR with a frequency of 1 in 5000 births. The majority of cases are terminal deletions that should also be detected by the telomeric probemixes P036 and P069/P070. Several interstitial deletions and complex rearrangements have been described.
- More info on monosomy 1p36 syndrome in OMIM 607872.

"2p16.1 microdeletion syndrome"

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
267	8386-L11411	FANCL (PHF9)	02-058.30	GACAAGAGCTGT-ATGCACTACCTC	2700 Kb
486	9860-L10628	REL	02-061.00	TCATGCCTCAAT-GGCACCTCTGCC	

- The interstitial 2p16.1 microdeletion syndrome has been described by Rajcan-Separovic E. et al (2007) J.Med.Genet. 44: 269-276. Phenotype includes moderate to severe mental retardation.
- For more 2p16 probes, enquire at info@mlpa.com.

"3q29 microdeletion syndrome"

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	2690-L02842	KIAA0226	P069/P070 probe for 3q. Located between the telomere and the microdeletion syndrome region.		126 Kb
	2013-L02052	BDH	P036 probe for 3q		250 Kb
418	8401-L08255	DLG1	03-198.51	CAGCTCAGAAGT-TCCATAGAACGG	231 Kb
359	8395-L08249	DLG1	03-198.28	CTATGAAAGACA-GGATAAATGATG	

- The interstitial 3q29 microdeletion syndrome has been described by Willatt, L. et al (2005) A.J.Hum.Genet. 77:154-160. Phenotype includes mild to moderate mental retardation. The P036 probe for the BDH gene is also located in the commonly deleted region. The P069-P070 probe is not, as it is located between the commonly deleted region and the telomere.
- For more 3q29 probes, enquire at info@mlpa.com.
- More info on the 3q29 microdeletion syndrome can be found in OMIM 609425.

Wolf-Hirschhorn syndrome, 4p16.3

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	2779-L02221	ZNF141	P069 / P070 probe for 4p		183 Kb
	2005-L02047	PIGG	P036 probe for 4p		1308 Kb
232	4190-L05920	LETM1	04-001.81	CCTGTGTACACA-TCCTCCAGAGGC	89 Kb
445	6058-L05513	WHSC1	04-001.90	GCTGAGTGAGAA-GCAGAGAGCACG	

- More probes for the Wolf-Hirschhorn region are present in the P096 MR2 probemix. Most frequent cause is a terminal deletion of 4p16.3 that can also be detected by the telomeric probemixes P036 and P069/P070.
- The WHS critical region is located approximately 1.9 Mb from the telomere and includes the WHSC1 gene.
- More info on WHS in OMIM 194190.

Cri du Chat region, 5p15

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	2791-L02233	LOC133957		P069 / P070 probe for 5p	109 Kb
	1723-L1327	PDCD6		P036 probe for 5p	968 Kb
436	3761-L02477	TERT	05-001.34	TCTTTCTTTTAT-GTCACGGAGACC	62 Kb
283	1126-L00684	CRR9 (=CLPTM1L)	05-001.40	CGACCTGGTCTT-GAATGTGGAAGA	

- More probes for the Cri du Chat region are present in the P096 MR2 probemix.
- Most frequent cause of the Cri du Chat syndrome is a terminal deletion of 5p15 that can also be detected by the telomeric probemixes P036 and P069/P070.
- Interstitial deletions have also been described (Zhang, X. et al; Am.J.Hum.Genet. 76:312-326, 2005). Some interstitial deletions might not be detected by the two 5p15.33 probes in this P245 probemix. More info in OMIM 123450.

Sotos syndrome, 5q35.3

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
154	2595-L08077	NSD1, exon 17	05-176.62	ACCCACCCACTG-TTATGCAGAACA	32 Kb
454	2600-L02071	NSD1, exon 22	05-176.65	GGAAAGACTGTT-TGCAAATGTGGA	

- More probes for the NSD1 gene are present in the P026 Sotos probemix. Deletion of the complete NSD1 gene is a frequent cause of Sotos syndrome and may result in a more severe phenotype.
- Frequency of complete gene deletions has been reported as 10% (United Kingdom) to 45 % (Japan) of all NSD1 mutations detected. More info on Sotos syndrome is in OMIM 117550.
- Distance from the NSD1 gene to the 5q telomeric probes in P036 and P069-70 is approximately 3950 Kb. Most common cause of Sotos syndrome are point mutations in the NSD1 gene that will not be detected by these MLPA probes.

Williams syndrome, 7q11.23

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
310	1333-L00876	ELN, exon 1	07-073.08	GGGGATAAAACG-AGGTGCGGAGA	28 Kb
364	1336-L00878	ELN, exon 20	07-073.11	TTTCCCGCTTT-GGTGTCGGAGTC	41 Kb
391	1337-L02333	LIMK1	07-073.15	TGTGGGACCTTT-ATCGGTGACGGG	

- More probes in the Williams-Beuren syndrome (WBS) region are present in the P064 MR1 probemix and the P029 Williams probemix. The majority (>90%) of the WBS patients have a 1.6 Mb deletion that includes the ELN and LIMK1 genes. Majority of smaller deletions include the ELN gene. More info in OMIM 194050.
- Besides deletions of the WBS region, some duplication has also been described, giving rise to the Williams-Beuren duplication syndrome (OMIM 609757).

Langer-Giedion syndrome, 8q24.12

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
401	3081-L07411	TRPS1	08-116.75	CTCTTTTTTGGT-GCTGCTGGTTTC	976 Kb
427	1108-L00679	EIF3S3	08-117.73	TTCCTGCCCCAA-AACTTAGGCAAG	1155 Kb to EXT1

- Most LGS patients have a microdeletion that includes the TRPS1 and EXT1 genes. More probes for the Langer-Giedion region are present in the P215 EXT1 probemix and the P228 LGS probemix.
- More info in OMIM 150230.

"9q22.3 microdeletion syndrome"

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
319	4652-L04036	TGFBR1 exon 7	09-100.95	GTTCTCGATGAT-TCCATAAATATG	1 Kb
409	4653-L10006	TGFBR1 exon 8	09-100.95	GATGGGTGAGAA-GGTACAAGATCA	

- An interstitial 9q22.3 microdeletion syndrome that includes the TGFBR1 gene has been described by Redon R. et al (2006) Eur.J.Hum.Genet. 14:759-67. Clinical phenotype includes mental retardation, macrocephaly, overgrowth and trigonocephaly.
- More probes for the TGFBR1 gene are present in the P148 probemix.

DiGeorge region 2, 10p15.1

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
136	7632-L07317	GATA3	10-008.14	GAGCAACGCAAT-CTGACCGAGCAG	2453 Kb
349	1232-L07388	Hs.538604	10-010.59	TGTAGACCACAT-GATGGAGATTTG	

- More probes for the 10p15 DiGeorge region 2 are in the P250 DiGeorge probe set. More info in OMIM 601362.
- Besides this DGS2 region, deletion of the 17p terminal region can also cause a DiGeorge like phenotype. These 17p deletions should be detected by the P036 and P069/P070 telomere probe mixes. The great majority of DiGeorge syndrome patients have a 22q11 deletion.

Beckwith-Wiedemann syndrome, 11p15.5: No probes for the Beckwith-Wiedemann region are included, as the great majority of cases are due to methylation changes / uniparental disomy. Copy number changes and methylation changes in the BWS region can be detected by the ME030 BWS probemix. More info in OMIM 130650.

WAGR syndrome, 11p13

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
220	3253-L02690	PAX6, exon 5	11-031.78	GTGAATCAGCTC-GGTGGTGTCTTT	

- Most patients with WAGR syndrome have a chromosomal deletion that includes the PAX6 and WT1 genes.
- More probes for the PAX6 gene / WAGR region are present in the P219 PAX6 probemix and the P118 WT1 probemix. More info in OMIM 194072. Aniridia is an easy to detect feature of the WAGR syndrome phenotype.

Prader-Willi / Angelman syndrome, 15q12

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	1733-L01317	CYFIP1		P036B probe for "15p"	842 Kb
	7291-L08858	MKRN3		P036C-P036D probe for "15p"	120 Kb
214	6282-L01542	NDN	15-021.48	ACACTGCTGCGA-GGGTAGTGGGCA	1170 Kb
247*	2026-L10004	SNRPN	15-022.65	CATGGTACAACCT-GCGCTTGCGCAA	112 Kb
292	1318-L07970	SNRPN	15-022.76	GATTCTCGCTA-CTCCAATATGGC	403 Kb
160	4620-L00863	UBE3A	15-023.17	TCTTCTCAAGG-ATAGGTGATAGC	

* ‡ The 247 nt probe is located in a 28 Kb area that is often (1/75) deleted in the Askenazi Jew population, apparently without phenotypic effect: Buiting K. et al (1999) Am.J.Hum.Genet. 65:1588-1594.

- More probes for the Prader-Willi / Angelman region, including probes for the detection of methylation changes, are present in the ME028 probemix.
- The 214 nt NDN probe detects the same sequence as the 418 nt NDN probe in the P070 telomere probeset. The majority of the Prader-Willi and Angelman patients have a copy number change of the 15q12 region that should be detected by this P245 probemix. A considerable number of patients however (~ 30%) have a methylation change that can be detected by the ME028 probemix, but not with this P245 probemix. More info on Prader-Willi syndrome in OMIM 176270. More info on Angelman syndrome in OMIM 105830.

"15q24 deletion syndrome"

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
190	8380-L10003	SEMA7A exon 8	15-072.50	CTGGTATGCAGT-GATGCTGCCACC	307 Kb
325	6811-L06406	CYP1A1, exon 2	15-072.80	GTCAACCTGAAT-AATAATTTCTGGG	

- The 15q24 microdeletion syndrome has been described by Sharp, AJ et al (2007) Hum.Mol.Genet. 16: 567-572. Phenotype includes mental retardation and growth retardation.
- More 15q24 probes, as well as probes for the PML gene which is located very close to this 15q24 microdeletion syndrome region, are present in the P297 probemix

Rubinstein-Taybi syndrome, 16p13.3

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
172	3087-L02487	CREBBP	16-003.87	AGCAGGTGAAAA-TGGCTGAGAACT	

- More probes for the CREBBP gene are present in the P313 CREBBP probemix. Only a minority of Rubinstein-Taybi patients (10 %?) can be detected with the use of this single probe, as most patients have point mutations in the CREBP or EP300 genes. More info in OMIM 180849.
- The 16p13.3 deletion syndrome (OMIM 610543) is caused by larger deletions that include the CREBBP gene.

Miller-Dieker region, 17p13.3

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
142	4120-L03532	PAFAH1B1, ex 7	17-002.51	TGTAGGCACTCT-ATAGATCAAGCT	2 Kb
238	1443-L08394	PAFAH1B1, ex 3	17-002.52	CCAGAAAAATAT-GCATTGAGTGGT	

- More probes for PAFAH1B1 and other genes in the Miller-Dieker region are present in the P061 Lissencephaly.
- More info in OMIM 607432 (Lissencephaly 1) and 247200 (Miller-Dieker syndrome).
- Approximately 15 % of lissencephaly patients and 90 % of Miller-Dieker patients have a chromosomal deletion that includes the PAFAH1B1 gene.

Smith-Magenis syndrome, 17p11.2

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
465	9440-L11412	RAI1	17-017.53	GGCTCCGAGAGA-CGAGTGGGAGAG	306 Kb
274	1452-L00936	LRRC48	17-017.83	CGGATCTCCAAG-ATCGACTCCCTG	245 Kb
303	1453-L08499	LLGL1	17-018.08	CAGCAGTCTGCA-TCTCTGGGAGAT	

- More probes for the Smith-Magenis region are present in the P064 MR1 probemix. More info in OMIM 182290.
- Smith-Magenis syndrome is caused in most cases (90%) by a 3.7 Mb interstitial deletion. A milder phenotype is associated with a duplication of the same region.

NF1 microdeletion syndrome, 17q11.2

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
260*	3778-L11180	NF1, exon 12	17-026.56	TTGGTGAAACAC-TTCATAAAGCAG	21 Kb
335	2508-L02620	NF1, exon 20	17-026.58	CAAGCAACAAAG-CTAATCCTTAAC	

- The 260* nt probe has been more variable in some experiments. It will probably be replaced in the next lot.
- More probes for the NF1 gene are present in the P081 & P082 NF1 probemixes. More probes for other genes in this area are present in the P122 NF1 area probemix. More info in OMIM 162200.
- Approximately 5-20% of all NF1 patients carry a heterozygous deletion of approximately 1.5 Mb that includes the NF1 gene. This NF1 microdeletion results in a more severe phenotype that often includes mental retardation, facial dysmorphism and developmental delay.

"17q21.31 microdeletion syndrome"

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
472	7859-L07620	CRHR1, exon 8	17-041.26	TGACCAACTTCT-TCTGGATGTTTCG	180 Kb
226	7856-L08385	MAPT, exon 11	17-041.44	TAGCAACGTCCA-GTCCAAGTGTGG	8 Kb
342	7857-L08501	MAPT, exon 13	17-041.45	TCCAGTCGAAGA-TTGGGTCCCTGG	

- More probes for the MAPT gene and other genes in this 17q21.31 microdeletion area are present in the P275 MAPT-17q21 probemix. More info on this recently discovered microdeletion syndrome in OMIM 610443.
- The cause of the phenotype is suspected to be a deletion of the MAPT and CHFR1 genes. A duplication of the same region has recently been described in a girl with severe psychomotor developmental delay, facial dysmorphism and microcephaly (Kirchhoff, M. et al. (May 18, 2007) Eur.J.Med.Genet. Epub).

DiGeorge syndrome, 22q11.21

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	2725-L00660	IL17R		P070 probe for 22q11	647 Kb
	1740-L01310	BID		P036 probe for 22q11	1015 Kb
196	1218-L06270	CLDN5, AB-region	22-017.89	TTCGCCAACATT-GTCGTCCGCGAG	200 Kb
208	5464-L10114	GP1BB, AB-region	22-018.09	CACAACCGAGCT-GGTGCTGACCGG	1480 Kb
373	1235-L00773	SNAP29, CD-region	22-019.57	AGGAGCAAGATG-ACATTCTTGACC	

- More probes in the 22q11 DiGeorge region are present in the P250 DiGeorge probemix. More info in OMIM 188400.
- Deletions in 22q11 are the most frequent cause of DiGeorge syndrome. These 22q11 deletions can be variable in size. The majority (88 %?) include the AB, BC and CD regions, though some deletions are smaller (AB only) or larger.
- Cat eye syndrome patients can be detected with the probes in the P036 and P070 telomere probe sets, but not by the probes in this P245 mix.

Phelan-McDermid syndrome, 22q13

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
	2707-L00661	ARSA	P069 / P070 probe for 22q13		77 Kb
253	10181-L11410	SHANK3	22-049.49	GGTCGGACACAA-GCAGGTGGTGGC	17 Kb
382	10182-L11174	SHANK3	22-049.50	ACCAACTGTGAT-CAGTGAGCTCAG	45 Kb
	1762-L08761	RABL2B	P036 probe for 22q13		

- More probes in this Phelan-McDermid region are present in the P188 22q13 probemix. More info in OMIM 606232.
- The SHANK3 gene is suspected to be responsible for at least part of the phenotype. The RABL2B probe in P036 is located between SHANK3 and the 22q telomere. The SHANK3 probes are present in P245 from lot 1107 onwards.

X chromosome copy number changes

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
297	1412-L01059	DMD, exon 35	X-032.29	CATCAAGTCAT-TCTTTGGAGCGG	

- This DMD probe is not intended to detect DMD defects. Deletions / duplications of exon 35 are rare.
- This X chromosome probe can however be used to distinguish MECP2 duplications from X chromosome copy number alterations.

RETT syndrome, MECP2 duplication syndrome, Xq28

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
202	3409-L02797	MECP2, exon 1	X-153.02	CATTAATCCTTA-ACATTCAAATTC	67 Kb
148	9310-L09999	MECP2, exon 4	X-152.95	TTTCATCTCCA-TGCCAAGGCCAA	5 Kb
184	9311-L10002	MECP2, exon 4	X-152.94	CAGTAACACATA-GACTGTGCGCAT	

- More probes for the MECP2 gene are present in the P015 RETT probemix. More probes in the Xq28 region are present in the P049 Xq28 probemix. Distance to the Xqter probe in P069 / P070 is 1809 Kb. Distance to the Xqter probes in P036 is 1764 Kb.
- Please note that the P036 and P069 / P070 probes are in the pseudoautosomal region and are thus also present on Yqter. Deletions in the MECP2 result in RETT syndrome. A duplication of this gene appears to be a relatively frequent cause of mental retardation.

Chromosome Y probes

Length (nt)	SALSA MLPA probe	Gene	MV36	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
118	S0003-L00313	DBY	Y-013.54	AAACGGCATGCT-ATCACAAGAAAG	561 Kb
108	S0001-L08073	UTY	Y-014.10	CTTCGGTAGCTT-AAGTCTTGCCT	

- These probes are identical to the chromosome Y-specific probes included in P036 and P069 / P070.

Note: Suggestions for improvement of our products are highly appreciated. Development of new probemixes that include more probes for syndromes with similar phenotype (prenatal or postnatal) is possible in collaboration with specialists. Please notify us of any mistakes: info@mlpa.com.

Several of the syndromes included in this probemix have been discovered relatively recently. Please inform us of interesting results, or problems, obtained with this new product.

The OMIM website: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>

MV36 = 01-001.14 indicates that a probe is on chromosome 1, at 1.14 Mb distance from the P-telomere.

Please note that only part of the sequence detected by the probes is provided: 12 nt before and 12 nt after the ligation site of the MLPA probe. The complete sequence detected by the probes is between 52 and 73 nt. The complete sequences detected by the P245 probes are available upon request at info@mlpa.com. The Santa Cruz BLAT server, <http://genome.ucsc.edu/cgi-bin/hgBlat>, is a simple tool to obtain longer sequences surrounding the probe recognition sequence.

Finding the genetic cause of mental retardation with MLPA

The number of genes whose defect can result in mental retardation is very large. In some cases, particular phenotypic features suggest the involvement of a specific gene or chromosomal region. Numerous SALSA MLPA kits are available to find the cause of mental retardation with distinct (syndromic) features, such as RETT syndrome, Sotos syndrome and Prader Willi.

Unfortunately, for patients suffering from non-syndromic mental retardation, the genetic cause is found only in a minority of cases. Usually, primary screening of such patients is done by karyotyping or G-banding. When no abnormality is detected by these methods, we suggest screening the patients with the following two SALSA MLPA kits (see figure 1):

- SALSA MLPA kit *P245 Microdeletion syndromes* contains probes for 21 different microdeletion syndromes causing mental retardation. For more information, please see the P245 product description.
- SALSA MLPA kit *P036 Human telomere* contains one probe for each subtelomeric region and is designed to detect deletions/duplications of each subtelomeric region. Several studies have indicated that 3-8 % (see references p.2) of all cases of mental retardation is caused by aberrant copy numbers of subtelomeric regions.

SALSA MLPA kit P245 Microdeletion syndromes

In case an abnormality is found with the SALSA MLPA kit *P245 Microdeletion syndromes*, we recommend further investigation of the deletion or duplication with one of the microdeletion follow-up kits (see pages 5-10)

SALSA MLPA kit P036 Human telomere

When used correctly, MLPA will exclude the presence of abnormal copy numbers of subtelomeric regions in the majority of samples. In case an abnormality is detected by P036, there are three ways of confirming the result:

1. Using a SALSA MLPA telomere follow-up kit. Follow-up kits contain more probes per telomere (see table below) and are suitable to examine a specific region more closely.
2. Using SALSA MLPA kit *P069* or *P070 Human telomere* for independent confirmation of the results. Since these probemixes also contain one probe for each subtelomere, they offer a broad subtelomeric screening similar to the P036.
3. Confirmation by another method such as FISH.

Which option to choose depends on the resources available. The advantage of option 2 (broad screening with P069 or P070) is that a single probemix suffices to confirm all P036 probes. However, generally speaking, option 1 will offer the best screening. In most cases where you will find an abnormality with the P036, it will be a single probe that is affected. Using one of the detailed follow-up kits will not only allow you to confirm the actual presence of the aberration, but also to determine the length of a possible deletion/duplication.

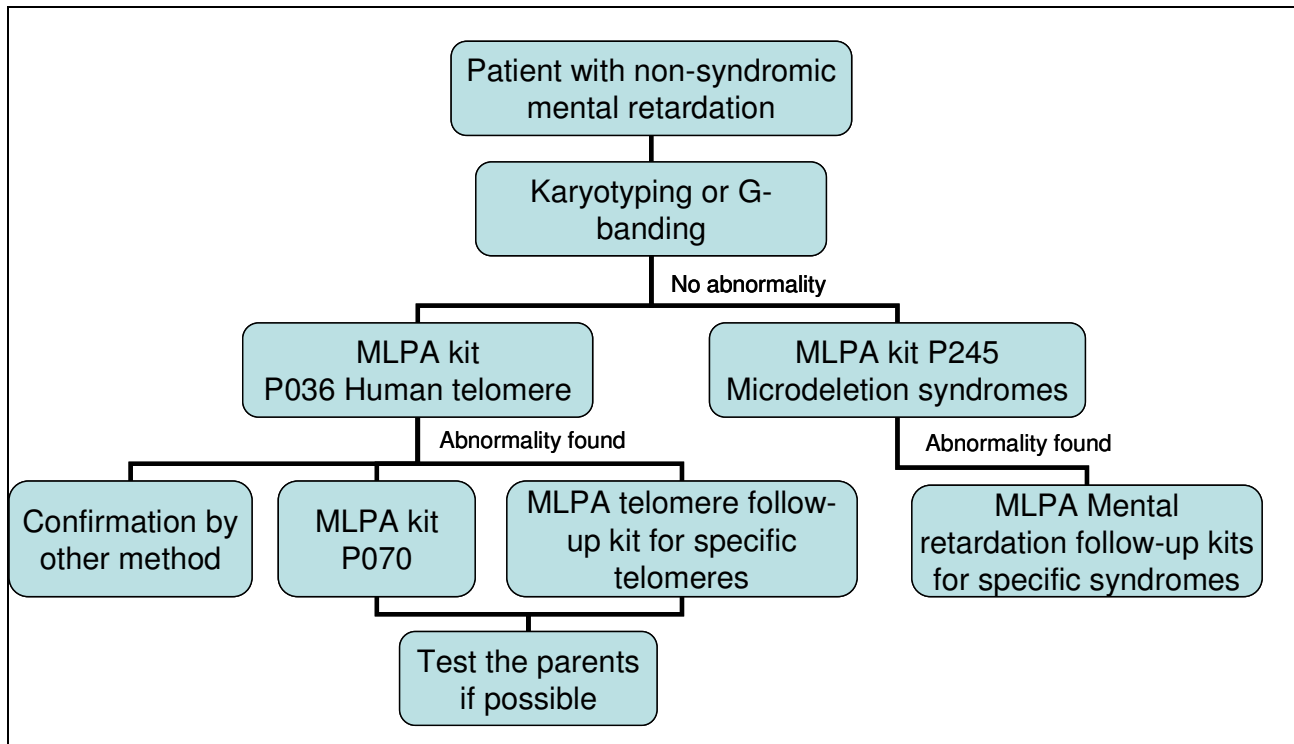


Figure 1: Flow scheme suggesting how to test a patient with non-syndromic mental retardation.

SALSA MLPA kit P245-A1 Microdeletion syndromes sample picture

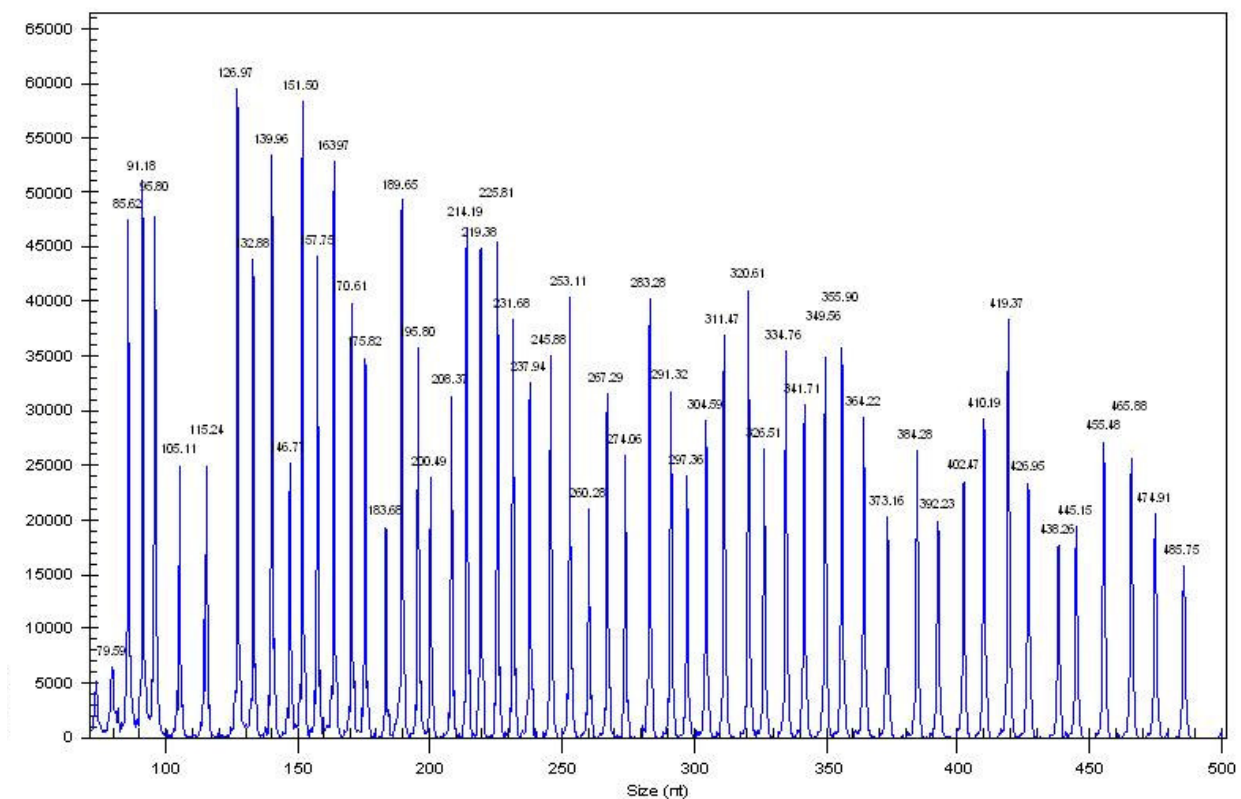


Figure 2. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P245-A1 Microdeletion syndromes (lot 1207).