SALSA MLPA probemix P061-D1 Lissencephaly

Lot D1-0117. As compared to previous version C1-0113, two probes targeting FLNA have been removed, one reference probe has been replaced and one removed, in additional several lengths of probes have been adjusted.

Classical lissencephaly, or isolated lissencephaly sequence (ILS), and subcortical band heterotopia (SBH) are neuronal migration disorders associated with severe mental retardation and epilepsy. Abnormalities of the PAFAH1B1 (LIS1) and DCX genes are implicated in the majority of patients with these disorders and account for approximately 75% of patients with ILS, whereas mutations of DCX account for 85% of patients with SBH. Lissencephaly may be associated with other diseases including Miller-Dieker syndrome, and Walker-Warburg syndrome. Duplications of the 17p13 region encompassing PAFAH1B1 have been reported to result in mild to moderate developmental delay.

This probemix includes probes for the lissencephaly related genes, such as; PAFAH1B1 (LIS1), DCX (SBH), POMT1 (Walker-Warburg syndrome), POMGnT1 (Muscle-Eye-Brain disease) and FLNA (periventricular nodular heterotopia, frontometaphyseal dysplasia and otopalatodigital syndrome).
The PAFAH1B1 gene (11 exons) spans ~92 kb of genomic DNA and is located on chromosome 17p13.3, ~2.5 Mb from the p-telomere. The P061-D1 probemix contains one probe for each exon of the PAFAH1B1 gene. Two probes are included for exon 1 and 2. Additionally, 8 probes flanking PAFAH1B1 are included.
The DCX gene (7 exons) spans ~118 kb of genomic DNA and is located on chromosome Xq23, ~111 Mb from the p-telomere. This probemix contains 8 probes for all exons of the DCX gene with the exception of exon 1. Two probes are included for exons 2 and 3.
The POMT1 gene (20 exons) spans ~21 kb of genomic DNA and is located on chromosome 9q34.13, ~131.5 Mb from the p-telomere. The P061-D1 probemix contains 4 probes for the POMT1 gene.
The POMGnT1 gene (23 exons) spans ~10 kb of genomic DNA and is located on chromosome 1p34.1, ~46 Mb from the p-telomere. The P061-D1 probemix contains 4 probes for the POMGnT1 gene.
The FLNA gene (48 exons) spans ~26 kb of genomic DNA and is located on chromosome Xq28, ~154 Mb from the p-telomere. This probemix contains 6 probes for the FLNA gene.
In addition, 8 reference probes are included in this probemix, detecting several different autosomal chromosomal locations.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned genes in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Note that a mutation or polymorphism (e.g. SNP) 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.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products 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

- P106 MRX: Contains more probes for X-linked mental retardation.

References for SALSA® MLPA® probemix P061 Lissencephaly

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Data analysis

The P061-D1 Lissencephaly probemix contains 51 MLPA probes with amplification products between 124 and 502 nt. In addition, the probemix 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, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Please note that the DCX and FLNA genes are located on the X-chromosome. Male samples should therefore be compared to male reference samples and female samples to female reference samples.

Data generated by this probemix can be normalised intra-sample by dividing the peak height of each amplification product by the total peak height of only the reference probes in the probemix (block normalisation). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes that no changes occurred in the genomic regions targeted by the reference probes. It is strongly recommended to use 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.

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.

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

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. For example, a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

This probemix was developed at MRC-Holland.

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

Table 1. SALSA MLPA P061-D1 Lissencephaly probemix

Length (nt)	SALSA MLPA probe	Chromosomal position				
		reference	PAFAH1B1	DCX	FLNA	other
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					
124	Reference probe 15370-L13762	7q11				
130 «	**FLNA probe** 10768-L11372				Exon 25	
136	**DCX probe** 10765-L11369			Exon 5		
142	**PAFAH1B1** probe 04120-L03532		Exon 3			
148 ¬	**TRPV1 probe** 01472-L00946		Downstream			
155	**POMT1 probe** 04128-L03485					Exon 2
160 ¬	**YWHAE probe** 04119-L03531		Upstream			
166	**DCX probe** 04122-L03479			Exon 2		
172 ¬ «	**KIAA0664 probe** 04610-L00948		Downstream			
176 ¥	**PAFAH1B1 probe** 20727-L28616					
184 ¬ «	**HIC1 probe** 10769-L11373		Upstream			
190 «	**FLNA probe** 04135-L03492				Exon 4	
196	**PAFAH1B1 probe** 07526-L06899		Exon 9			
203 «	**POMGnT1 probe** 04142-L24185					Exon 7
210	**DCX probe** 04123-L03480			Exon 3		
217 ¬ « ¥	**HIC1 probe** 21184-L00949		Upstream			
227	Reference probe 16643-L19177	3p21				
232	**PAFAH1B1 probe** 04605-L12936		Exon 6			
238 «	**PAFAH1B1 probe** 10770-L12010		Exon 1			
247	**PAFAH1B1 probe** 01926-L01478		Exon 8			
257 «	**FLNA probe** 04136-L03493				Exon 11	
266 ¥	**PAFAH1B1 probe** 21183-L11965		Exon 2			
275	**DCX probe** 04124-L03481			Exon 4		
283 «	**PAFAH1B1 probe** 01925-L01477		Exon 11			
292 «	**POMGnT1 probe** 04606-L03500					Exon 18
300 «	**PAFAH1B1 probe** 01927-L01479		Exon 1			
309	Reference probe 14153-L15753	6p25				
314	**PAFAH1B1 probe** 07276-L24293		Exon 2			
319	**DCX probe** 10763-L24057			Exon 2		
326	**POMT1 probe** 04132-L03489					Exon 17
333 *	Reference probe 18492-L24579	3p13				
340	**PAFAH1B1 probe** 04176-L24059		Exon 7			
346 ¬	**RAP1GAP2 probe** 00689-L14552		Downstream			
355 ¬	**YWHAE probe** 04118-L03530		Upstream			
360	Reference probe 05762-L05200	12q12				
366	**DCX probe** 10766-L24060			Exon 6		
373	**POMT1 probe** 04133-L03490					Exon 19
386	**PAFAH1B1 probe** 10772-L24349		Exon 4			
391 «	**FLNA probe** 17262-L21149				Exon 38	
401 «	**FLNA probe** 04608-L24347				Exon 22	
409	**DCX probe** 04127-L08388			Exon 7		
425 ‡ «	**PAFAH1B1 probe** 10774-L24345		Exon 10			
429 ¬	**METTL16 probe** 01924-L15414		Upstream			
436	**POMT1 probe** 04129-L03486					Exon 5
444	Reference probe 05916-L24543	21q11				
451 «	**FLNA probe** 04138-L15416				Exon 29	
456 «	**POMGnT1 probe** 04141-L24724					Exon 3
463 «	**POMGnT1 probe** 04609-L24723					Exon 21
483	**DCX probe** 10764-L11368			Exon 3		
494	Reference probe 14882-L17578	14q11				
502	Reference probe 06676-L23439	11p15				

* New in version D1 (from lot D1-0117 onwards).
¥ Changed in version D1 (from lot D1-0117 onwards). Small change in length, no change in sequence detected.
« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition being tested.
‡ A SNP (rs1803915) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

Table 2. P061 probes arranged according to chromosomal location

Table 2a. DCX gene

Length (nt)	SALSA MLPA probe	DCX exon	Ligation site NM_178152.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*118-120 (exon 2)*		
	No probe	Exon 1			
319	10763-L24057	Exon 2	559 nt before exon 2; 167-168 in NM_000555.3	AGAGGGCTTGGA-ATAAAATGAAAA	0.7 kb
166	04122-L03479	Exon 2	268-269	GTAATGAGAAGA-AAGCCAAGAAGG	9.0 kb
483	10764-L11368	Exon 3	561-562	AACTGGTCTGTC-AACGTAAAAACA	0.2 kb
210	04123-L03480	Exon 3	756-757	GTCCTCACTGAT-ATCACAGAAGCC	67.9 kb
275	04124-L03481	Exon 4	864-865	GATGATGTGTTT-ATTGCCTGTGGT	2.2 kb
136	10765-L11369	Exon 5	1058-1059	AGGTAACGACCA-AGACGGTGAGTG	18.0 kb
366	10766-L24060	Exon 6	177 nt before exon 6	TTAACTGAGGAT-TGCAGTTCTTGC	11.2 kb
409	04127-L08388	Exon 7	1205-1206	CTCGCTTGGTGA-TTCCATGTAAAG	
		Stop codon	*1213-1215 (exon 7)*		

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

Table 2b. FLNA gene

Length (nt)	SALSA MLPA probe	FLNA exon	Ligation site NM_001110556.1	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*250-252 (exon 2)*		
190 «	04135-L03492	Exon 4	915-916	AGCAAGCCCGTT-ACCAATGCGCGA	2.5 kb
257 «	04136-L03493	Exon 11	1875-1876	GGCTTCGAGTAT-TACCCCATGGTC	5.1 kb
401 «	04608-L24347	Exon 22	3905-3906	CACGCACACCAT-TACCTACATTCC	0.8 kb
130 «	10768-L11372	Exon 25	4513-4514	AGGCTGGCACCT-ACAGCCTCAACG	1.7 kb
451 «	04138-L15416	Exon 29	5060-5061	TGACGGCACGTA-TACAGTGGCCTA	4.5 kb
391 «	17262-L21149	Exon 38	6452-6453	GCCTGCAGAGTT-TATCATTGATAC	
		Stop codon	*8191-8193 (exon 48)*		

Note: The NM_001110556.1 sequence represents transcript variant 2 and is a reference standard in the NCBI RefSeqGene project.

Table 2c. POMGnT1 gene

Length (nt)	SALSA MLPA probe	POMGnT1 exon	Ligation site NM_017739.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*165-167 (exon 3)*		
456 «	04141-L24724	Exon 3	233-234	AGCTGGTACCTT-ACCTGGAAGTAT	1.9 kb
203 «	04142-L24185	Exon 7	650-651	GCCATGGTGCTA-TTCCTCAACATG	3.7 kb
292 «	04606-L03500	Exon 18	1666-1667	ATCCTACCACTT-TGGCATCGTCGG	2.2 kb
463 «	04609-L24723	Exon 21	1905-1906	CCTTTATTCGAA-TGGAGAAAGATG	
		Stop codon	*2145-2147 (exon 23b)*		

Note: The NM_017739.3 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Table 2d. POMT1 gene

Length (nt)	SALSA MLPA probe	POMT1 exon	Ligation site NM_007171.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*203-205 (exon 2)*		
155	04128-L03485	Exon 2	248-249	CGGCTGACATCA-ACTTGAGCCTTG	3.2 kb
436	04129-L03486	Exon 5	585-586	GGAGCTCCACTT-TTCTCATTGTGC	12.6 kb
326	04132-L03489	Exon 17	1887-1888	TGATGACTCGGA-ACACAAGTACAG	2.1 kb
373	04133-L03490	Exon 19	2220-2221	ACTCACCTTCCA-AATCCTTCTGCT	
		Stop codon	*2444-2446 (exon 20)*		

Note: The NM_007171.3 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Table 2e. PAFAH1B1 gene (17p13.3, Miller-Dieker Region)

Length (nt)	SALSA MLPA probe	Exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
160 ¬	04119-L03531	YWHAE (ex 5)		GCCACAGGAAAC-GACAGGAAGGAG	38.9 kb
355 ¬	04118-L03530	YWHAE (ex 1)		CCGCTGCCGCTA-TGGATGATCGAG	655.0 kb
217 ¬ « ¥	21184-L00949	HIC1 (ex 1)		AGAGTGTGCGGA-AAGCGCGGCGGG	2.8 kb
184 ¬ «	10769-L11373	HIC1 (ex 3)		AGCCCGAGAGCT-TCGGTGACAACC	453.9 kb
429 ¬	01924-L15414	METTL16		CGGCTGCTTTAA-GATTCTAGGGTT	81.6 kb
		PAFAH1B1	**NM_000430.3**		
		Start codon	*569-571 (exon 2)*		
300 «	01927-L01479	Exon 1	213 nt before exon 1	TAACAGAAGCGT-GCGGAGCGTGAG	0.3 kb
238 «	10770-L12010	Exon 1	112-113	ACACGGGAGTCT-AGGGAGCGAGAA	44.4 kb
265 ¥	21183-L11965	Exon 2	454-455	ATTTTCCCCTGT-GTGGAAGACACT	0.1 kb
314	07276-L24293	Exon 2	543-544	TACCACTATATC-AGATAAGCTTGA	26.8 kb
142	04120-L03532	Exon 3	309 nt before exon 3, reverse	TGTAGGCACTCT-ATAGATCAAGCT	1.2 kb
386	10772-L24349	Exon 4	183 nt after exon 4	GGTAATTCACAT-ATCTGGAGTTGC	0.8 kb
176 ¥	20727-L28616	Exon 5	874-875	CCAGAAAAATAT-GCATTGAGTGGT	3.2 kb
232	04605-L12936	Exon 6	1080-1081	CTGTTCTGCAGA-TATGACCATTAA	2.4 kb
340	04176-L24059	Exon 7	1176-1177	CATCATGCCCAA-TGGAGATCATAT	1.5 kb
247	01926-L01478	Exon 8	1367-1366 reverse	AGCCTTGCATTC-CTTTGTTGCTAC	2.4 kb
196	07526-L06899	Exon 9	1516-1517	TCTGGATCCAGA-GACAAGACTATT	3.6 kb
425 « ‡	10774-L24345	Exon 10	1607-1606 reverse	CCCCCCAGAATG-GAACAGAACTCC	1.6 kb
283 «	01925-L01477	Exon 11	1768-1769	ACTGGCAGCGTA-GATCAAACAGTA	13.3 kb
		Stop codon	*1799-1801 (exon 11)*		
172 ¬ «	04610-L00948	KIAA0664		AACTGCTTCCTG-AGCTCCTACCCA	303.2 kb
346 ¬	00689-L14552	RAP1GAP2		CTTTTTATTTAG-CTCCAGAGAAAG	568.6 kb
148 ¬	01472-L00946	TRPV1		CAGCCCGAGGAA-GTTTATCTGCGA	

¥ Changed in version C1 (from lot D1-0117 onwards). Small change in length, no change in sequence detected.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition being tested.
« This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
‡ A SNP (rs1803915) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Note: The NM_000430.3 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 P061-D1 Lissencephaly sample pictures

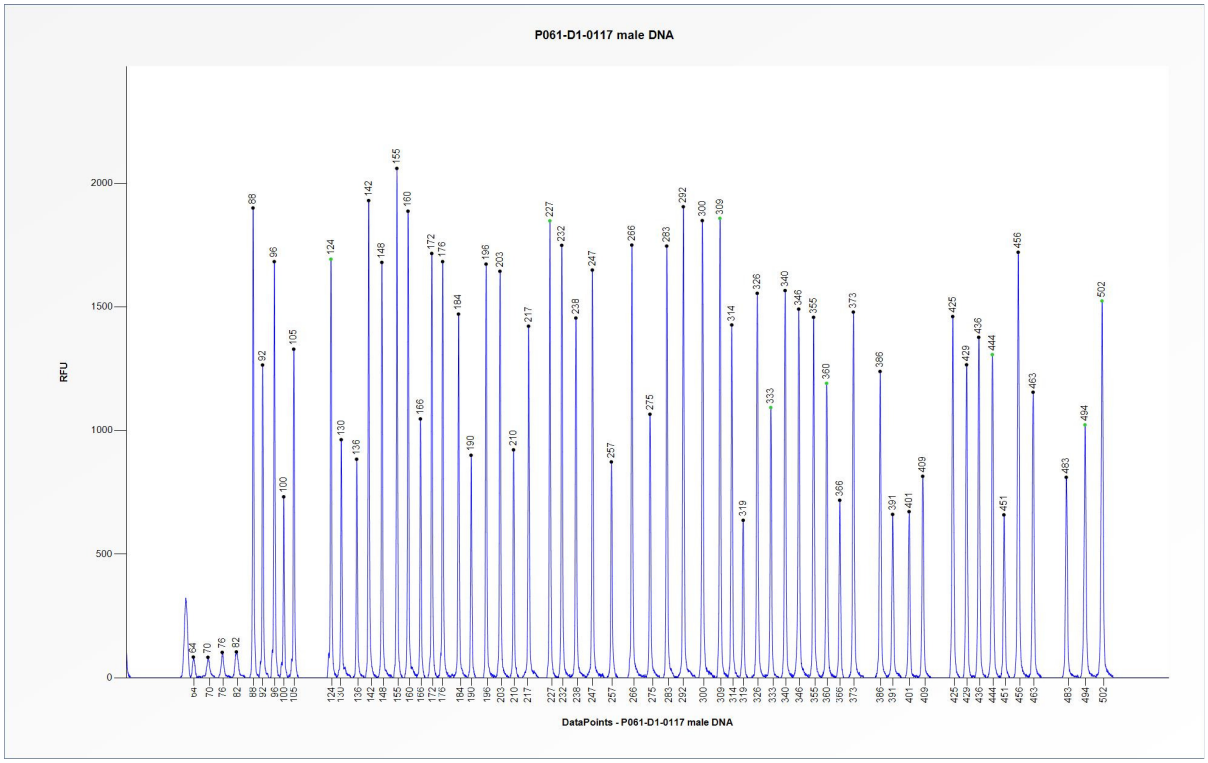

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P061-D1 Lissencephaly (lot D1-0117).

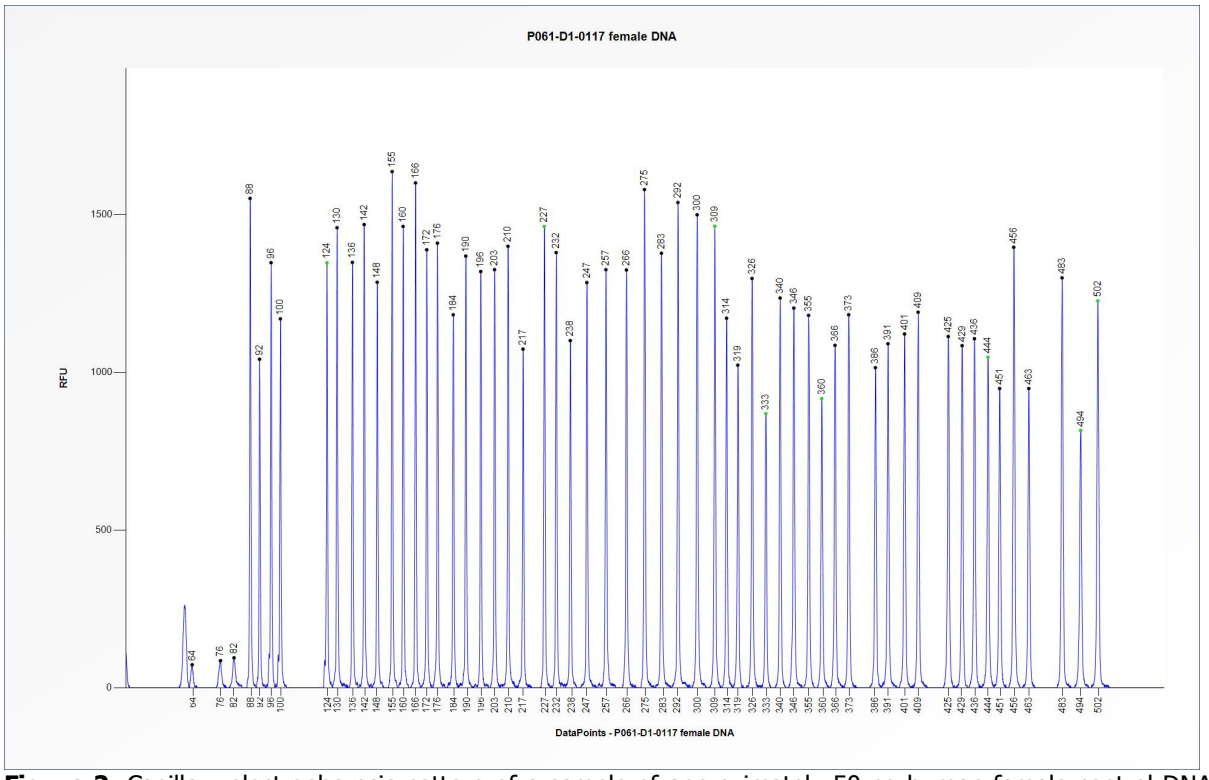

Figure 2. Capillary electrophoresis pattern of a sample of approximately 50 ng human female control DNA analysed with SALSA MLPA probemix P061-D1 Lissencephaly (lot D1-0117).

Implemented Changes – compared to the previous product description version(s).

Version 13 – 31 January 2017 (55)
- Product description adapted to a new product version (version number changed, lot number added, new pictures included).
- Salt-sensitivity warning added for several probes in Table 1 and 2.
- References added.

Version 12 – 10 January 2017 (55)
- Warning added in Table 1, 172 nt probe 04610-L00948, 184 nt probe 10769-L11373, 203 nt probe 04142-L24185, 220 nt probe 03804-L00949, 472 nt probe 04140-L03497.

Version 11 – 29 February 2016 (55)
- DCX exon numbering adjusted in Table 1 and 2a.

Version 10 (53)
- Warning added in Tables 1 and 2 on the FLNA exon 39 probe being insufficiently specific.

Version 09 (49)
- Product description adapted to a new product version (version number changed, lot number added, new pictures included).
- Various textual changes on page 1 and 2.
- Various layout changes.
- Changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 08 (48)
- Warning added in Table 1, 172 nt probe 04610-L00948; 202 nt probe 04142-L03499; 454 nt probe 04141-L03498.

Version 07 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 06 (48)
- Ligation sites 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; tables have been numbered.
- Remark on RefSeqGene standard and transcript variant added below Table 2.
- Various minor textual changes on page 1.
- The gene name of METT10D has changed into METTL16.

Version 05 (46)
- Sample pictures have been exchanged. For more information see figure 1 and 2.
- Correction on page 1 regarding the number of control fragments added in version B2.
- Related probemix paragraph added on page 1.
- Warning added in Table 1, 184 nt probe 10769-L11373, 220 nt probe 03804-L00949, and 418 nt probe 10774-L12012.

Version 4 (45)
- 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).
- Exon numbering of the DCX, FLNA and POMGnT1 genes has been changed in Tables 1 and 2.
- Warning added below Tables 1 and 2 that the exon numbering used for some genes is new.
- Ligation sites 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; tables have been numbered.
- Data analysis method has been modified; sentence "when only small numbers of samples are tested, visual comparison of peak profiles should be sufficient" removed from data analysis section