Product Description SALSA® MLPA® Probemix P061-D2 Lissencephaly

To be used with the MLPA General Protocol.

Version D2. As compared to version D1, three reference probes have been added and two reference probes have been replaced. In addition, two probe lengths have been adjusted. For complete product history see page 8.

Catalogue numbers:
- **P061-025R:** SALSA MLPA Probemix P061 Lissencephaly, 25 reactions.
- **P061-050R:** SALSA MLPA Probemix P061 Lissencephaly, 50 reactions.
- **P061-100R:** SALSA MLPA Probemix P061 Lissencephaly, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information: The SALSA MLPA Probemix P061 Lissencephaly is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PAFAH1B1*, *DCX*, *POMT1*, *POMGNT1* and *FLNA* genes, which is associated with Lissencephaly.

Classical lissencephaly (LIS1), or isolated lissencephaly sequence (ILS), and subcortical band heterotopia (SBH) are neuronal migration disorders associated with severe intellectual disability and epilepsy. Abnormalities of the *PAFAH1B1* 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 *DCX* gene (7 exons), spans ~118 kb of genomic DNA and is located on chromosome Xq23, ~111 Mb from the p-telomere.
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 *POMGNT1* gene (22 exons), spans ~10 kb of genomic DNA and is located on chromosome 1p34.1, ~46 Mb from the p-telomere.
The *FLNA* gene (48 exons), spans ~26 kb of genomic DNA and is located on chromosome Xq28, ~154 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK5189.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering: The *PAFAH1B1*, *DCX*, *POMT1*, *POMGNT1* and *FLNA* exon numbering used in this P061-D2-0920 Lissencephaly product description is the exon numbering from the RefSeq transcript NM_000430.4, NM_178152.3, NM_007171.3 (LRG_842), NM_017739.4 (LRG_701) and NM_001110556.2 (LRG_1340), which are identical to the NG_009799.1, NG_011750.1, NG_008896.1 NG_009205.2 and NG_011506.2 sequences respectively. The exon numbering and NM_ sequence used have been retrieved on 10/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P061-D2 Lissencephaly contains 54 MLPA probes with amplification products between 124 and 503 nucleotides (nt). This includes: one probe for each exon of the *PAFAH1B1* gene, two probes are included for exon 1 and 2; eight probes flanking *PAFAH1B1* are included, five upstream and three downstream; eight probes for all exons of the *DCX* gene with the exception of exon 1, two probes are included for exons 2 and 3; four probes for the *POMT1* gene; four probes for the *POMGNT1* gene; six probes for the *FLNA* gene. In addition, eleven reference probes are included in P061-D2, that detects several chromosomal autosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals of the same sex is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of Lissencephaly. It is recommended to use samples of the same sex to facilitate

interpretation. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number NA06047 from the Coriell Institute has been tested with this P061 probemix at MRC-Holland and can be used as positive control samples to detect a heterozygous deletion of 17p (telomere-*TRPV1*). The quality of cell lines can change; therefore samples should be validated before use.

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results:

Copy number status		**Dosage quotient**
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < DQ < 1.20
Homozygous deletion	Deletion	DQ = 0
Heterozygous deletion		0.40 < DQ < 0.65
Heterozygous duplication		1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	Duplication	1.75 < DQ < 2.15
Ambiguous copy number		All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale,

rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *PAFAH1B1*, *DCX*, *POMT1*, *POMGNT1* and *FLNA* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P061 Lissencephaly.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***PAFAH1B1*, *DCX*, *POMT1*, *POMGNT1* and *FLNA* mutation database:** https://databases.lovd.nl/shared/genes/. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *DCX* exons 4 and 6 but not exon 5) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P061-D2 Lissencephaly

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*PAFAH1B1*	*DCX*	*FLNA*	other
64-105	Control fragments – see table in probemix content section for more information					
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		Exon 5			
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		
216 *	Reference probe 14288-L32317	15q13				
222 ¥ « ¬	**HIC1 probe** 21184-L32297		Upstream			
228	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				
315	**PAFAH1B1 probe** 07276-L24293		Exon 2			
319	**DCX probe** 10763-L24057			Exon 2		
326	**POMT1 probe** 04132-L03489					Exon 17
337 *	Reference probe 08322-L32318	2p23				
340	**PAFAH1B1 probe** 04176-L24059		Exon 7			
347 ¬	**RAP1GAP2 probe** 00689-L14552		Downstream			
355 ¬	**YWHAE probe** 04118-L03530		Upstream			
360	Reference probe 05762-L05200	12q12				
368	**DCX probe** 10766-L24060			Exon 6		
375	**POMT1 probe** 04133-L03490					Exon 19
382 *	Reference probe 18237-L32319	13q22				
388	**PAFAH1B1 probe** 10772-L24349		Exon 4			
393	**FLNA probe** 17262-L21149				Exon 38	
403 «	**FLNA probe** 04608-L24347				Exon 22	
409	**DCX probe** 04127-L08388			Exon 7		
420 *	Reference probe 15824-L17885	19q13				
426 ¥ ±	**PAFAH1B1 probe** 10774-L32320		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
465	**POMGNT1 probe** 04609-L24723					Exon 21
483	**DCX probe** 10764-L11368			Exon 3		
492 *	Reference probe 17001-L30500	20q11				
503	Reference probe 06676-L23439	11p15				

a) See above section on exon numbering for more information.

* New in version D2.
¥ Changed in version D2. Minor alteration, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition 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.

Table 2. P061 probes arranged according to chromosomal location

Table 2a. *DCX* gene

Length (nt)	SALSA MLPA probe	*DCX* exon[a]	Ligation site NM_178152.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*78-80 (exon 2)*		
	No probe	Exon 1			
319	10763-L24057	Exon 2	559 nt before exon 2	AGAGGGCTTGGA-ATAAAATGAAAA	0.7 kb
166	04122-L03479	Exon 2	228-229	GTAATGAGAAGA-AAGCCAAGAAGG	9.0 kb
483	10764-L11368	Exon 3	521-522	AACTGGTCTGTC-AACGTAAAAACA	0.2 kb
210	04123-L03480	Exon 3	716-717	GTCCTCACTGAT-ATCACAGAAGCC	67.9 kb
275	04124-L03481	Exon 4	824-825	GATGATGTGTTT-ATTGCCTGTGGT	2.2 kb
136	10765-L11369	Exon 5	1018-1019	AGGTAACGACCA-AGACGGTGAGTG	18.0 kb
368	10766-L24060	Exon 6	177 nt before exon 6	TTAACTGAGGAT-TGCAGTTCTTGC	11.2 kb
409	04127-L08388	Exon 7	1165-1166	CTCGCTTGGTGA-TTCCATGTAAAG	
		Stop Codon	*1173-1175 (exon 7)*		

Table 2b. *FLNA* gene

Length (nt)	SALSA MLPA probe	*FLNA* exon[a]	Ligation site NM_001110556.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*246-248 (exon 2)*		
190	04135-L03492	Exon 4	911-912	AGCAAGCCCGTT-ACCAATGCGCGA	2.5 kb
257	04136-L03493	Exon 11	1871-1872	GGCTTCGAGTAT-TACCCCATGGTC	5.1 kb
403	04608-L24347	Exon 22	3901-3902	CACGCACACCAT-TACCTACATTCC	0.8 kb
130	10768-L11372	Exon 25	4509-4510	AGGCTGGCACCT-ACAGCCTCAACG	1.7 kb
451	04138-L15416	Exon 29	5056-5057	TGACGGCACGTA-TACAGTGGCCTA	4.5 kb
393	17262-L21149	Exon 38	6448-6449	GCCTGCAGAGTT-TATCATTGATAC	
		Stop Codon	*8187-8189 (exon 48)*		

Table 2c. *POMGNT1* gene

Length (nt)	SALSA MLPA probe	*POMGNT1* exon[a]	Ligation site NM_017739.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*150-152 (exon 2)*		
456	04141-L24724	Exon 3	218-219	AGCTGGTACCTT-ACCTGGAAGTAT	1.9 kb
203	04142-L24185	Exon 7	635-636	GCCATGGTGCTA-TTCCTCAACATG	3.7 kb
292	04606-L03500	Exon 18	1651-1652	ATCCTACCACTT-TGGCATCGTCGG	2.2 kb
465	04609-L24723	Exon 21	1890-1891	CCTTTATTCGAA-TGGAGAAAGATG	
		Stop Codon	*2130-2132 (exon 22)*		

Table 2d. *POMT1* gene

Length (nt)	SALSA MLPA probe	*POMT1* exon[a]	Ligation site NM_007171.3	Partial sequence[b] (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
375	04133-L03490	Exon 19	2220-2221	ACTCACCTTCCA-AATCCTTCTGCT	
		Stop Codon	2444-2446 (exon 20)		

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

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
160	04119-L03531	*YWHAE* (ex 4)		GCCACAGGAAAC-GACAGGAAGGAG	38.9 kb
355	04118-L03530	*YWHAE* (ex 1)		CCGCTGCCGCTA-TGGATGATCGAG	655.0 kb
222	21184-L32297	*HIC1* (ex 1)		AGAGTGTGCGGA-AAGCGCGGCGGG	2.8 kb
184	10769-L11373	*HIC1* (ex 2)		AGCCCGAGAGCT-TCGGTGACAACC	453.9 kb
429	01924-L15414	*METTL16*		CGGCTGCTTTAA-GATTCTAGGGTT	81.6 kb
		PAFAH1B1	**NM_000430.4**		
		Start Codon	*544-546 (exon 2)*		
300	01927-L01479	Exon 1	238 nt before exon 1	TAACAGAAGCGT-GCGGAGCGTGAG	0.3 kb
238	10770-L12010	Exon 1	87-88	ACACGGGAGTCT-AGGGAGCGAGAA	44.4 kb
266	21183-L11965	Exon 2	429-430	ATTTTCCCCTGT-GTGGAAGACACT	0.1 kb
315	07276-L24293	Exon 2	518-519	TACCACTATATC-AGATAAGCTTGA	26.8 kb
142	04120-L03532	Exon 3	308 nt before exon 3, reverse	TGTAGGCACTCT-ATAGATCAAGCT	1.2 kb
388	10772-L24349	Exon 4	182 nt after exon 4	GGTAATTCACAT-ATCTGGAGTTGC	0.8 kb
176	20727-L28616	Exon 5	849-850	CCAGAAAAATAT-GCATTGAGTGGT	3.2 kb
232	04605-L12936	Exon 6	1055-1056	CTGTTCTGCAGA-TATGACCATTAA	2.4 kb
340	04176-L24059	Exon 7	1151-1152	CATCATGCCCAA-TGGAGATCATAT	1.5 kb
247	01926-L01478	Exon 8	1342-1341 reverse	AGCCTTGCATTC-CTTTGTTGCTAC	2.4 kb
196	07526-L06899	Exon 9	1491-1492	TCTGGATCCAGA-GACAAGACTATT	3.6 kb
426	10774-L32320	Exon 10	1582-1581 reverse	CCCCCCAGAATG-GAACAGAACTCC	1.6 kb
283	01925-L01477	Exon 11	1743-1744	ACTGGCAGCGTA-GATCAAACAGTA	13.3 kb
		Stop Codon	*1774-1776 (exon 11)*		
172	04610-L00948	*KIAA0664*		AACTGCTTCCTG-AGCTCCTACCCA	303.2 kb
347	00689-L14552	*RAP1GAP2*		CTTTTTATTTAG-CTCCAGAGAAAG	568.6 kb
148	01472-L00946	*TRPV1*		CAGCCCGAGGAA-GTTTATCTGCGA	

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P106 MRX: Contains more probes for X-linked intellectual disability.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.

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Selected publications using SALSA MLPA Probemix P061 Lissencephaly

- Herbst, S. M. et al. 2016. LIS1-associated classic lissencephaly: A retrospective, multicenter survey of the epileptogenic phenotype and response to antiepileptic drugs. *Brain and Development.* 38: 399-406.
- Lange, M. et al. 2015. 47 patients with FLNA associated periventricular nodular heterotopia. *Orph J of rare Dis.* 10: 1.
- Takahashi, S. et al. 2015 Characterization of intragenic tandem duplication in the PAFAH1B1 gene leading to isolated lissencephaly sequence. *Mol Cytogen.* 8:1.
- Clapham, K. R. et al. 2012. FLNA genomic rearrangements cause periventricular nodular heterotopia. *Neurology.* 78: 269-278.
- Haverfield, E. V. et al. 2009. Intragenic deletions and duplications of the LIS1 and DCX genes: a major disease-causing mechanism in lissencephaly and subcortical band heterotopia. *Eur J Genet.* 17:911-8.
- Mei, D. et al. 2008. High frequency of genomic deletions — and a duplication — in the LIS1 gene in lissencephaly: implications for molecular diagnosis. *J Med Genet.* 45:355-61.
- Mei, D. et al. 2007. Multiplex ligation-dependent probe amplification detects DCX gene deletions in band heterotopia. *Neurology.* 68:446-50.

P061 Product history	
Version	*Modification*
D2	Three reference probes have been added and two reference probes have been replaced. In addition, two probe lengths have been adjusted.
D1	Two probes targeting FLNA have been removed, one reference probe has been replaced and one removed, in addition several lengths of probes have been adjusted.
C1	One additional FLNA probe and nine reference probes have been included. The 118 nt Y-fragment has been removed and the control fragments have been adjusted (QDX2).
B2	Four extra control fragments at 88-96-100-105 nt and a 118 nt Y-specific probe have been added.
B1	First release.

Implemented changes in the product description

Version D2-02 – 11 December 2024 (02P)
- The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate.

Version D2-01 — 26 November 2020 (02P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *PAFAH1B1*, *POMGNT1* and *FLNA* genes updated according to new version of the NM_ reference sequences.
- *POMGnT1* has been renamed to *POMGNT1*

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.

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands

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Phone	+31 888 657 200