SALSA MLPA probemix P357-A3 MODY Mix 2

Lot A3-1217. As compared to the previous lot A2-0715, one reference probe has been replaced.

Maturity-Onset Diabetes of the Young (MODY) is a distinct form of non insulin-dependent diabetes mellitus (NDDM), also known as type II diabetes. MODY has a clear autosomal inheritance and it generally develops in individuals under 25 years of age. Approximately 5% of all diabetes patients suffer from MODY. As described in more detail on page 2, eleven forms of MODY have now been identified with the highest prevalence for MODY 2 and 3. Each form of MODY has been associated with one gene.

This P357-A3 MODY Mix 2 probemix contains probes for the PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4 and INS genes. This probemix is therefore specific for MODY 4-10. In addition, 10 reference probes are included in this probemix, detecting 10 different autosomal chromosomal locations.

The widely used probemix P241 MODY Mix 1 contains probes for the HNF4A, GCK, HNF1A and HNF1B genes involved in MODY types 1-3 and 5, the more frequent causes of MODY. For these genes a clear relation has been described between copy number changes of the genes and the MODY phenotype or the renal cysts and diabetes (RCAD) syndrome.

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 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).

Related SALSA® MLPA® probemixes

- P241 MODY Mix 1: Contains probes for HNF4A, GCK, HNF1A and HNF1B genes, involved in MODY 1, 2, 3 and 5, respectively.
- P117 ABCC8: Contains probes for the ABCC8 gene, involved in familial hyperinsulinemic hypoglycemia 1.
- ME033 TNDM: Contains probes for several genes involved in transient neonatal diabetes mellitus, including the INS gene.

More information

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The MODY genes

- **MODY 1** is a result of defects in the Hepatocyte nuclear factor-4-alpha (**HNF4A**) gene on chromosome 20q13. This gene comprises 12 exons and spans about 30 kb of genomic DNA. The HNF4A protein regulates the expression of HNF1A. MLPA probes for the HNF4A gene are present in the P241 probemix.
- **MODY 2** is caused by mutations in Glucokinase gene (**GCK**) on chromosome 7p13. This gene comprises 12 exons and spans about 45 kb of genomic DNA. MLPA probes for GCK are present in the P241 probemix.
- **MODY 3** is caused by defects in the HNF1 Homeobox A gene (**HNF1A**) which comprises 10 exons and spans 24 kb of genomic DNA on 12q24. MLPA probes for HNF1A are present in the P241 probemix.
- **MODY 4** has been linked to defects in the Pancreas/Duodenum homeobox protein 1 gene (**PDX1**) on chromosome 13q12.2. This gene comprises 2 exons and spans about 6 kb of genomic DNA.
- **MODY 5** have been associated with the HNF1 Homeobox B gene (**HNF1B**) on chromosome 17q12. The HNF1B gene comprises 9 exons and spans about 59 kb of genomic DNA. The HNF1B probes in this P357 probemix detect the same sequences as the HNF1B probes in P241.
- **MODY 6** has been linked to defects in the **NEUROD1** gene on 2q31 which has 2 exons spanning 4 kb.
- **MODY 7** is caused by mutations in the Kruppel-Like Factor 11 gene (**KLF11**) on chromosome 2p25.1. KLF11 gene consists of 6 exons and spans approximately 11 kb of genomic DNA.
- **MODY 8** has been associated with defects in Carboxyl-ester lipase gene (**CEL**), which comprises 11 exons spanning 10 kb on chromosome 9q34. Due to sequence similarity with the CEL pseudogene CELP, only probes for exons 2 to 7 of CEL are included. Compared to CEL, CELP lacks a 4.8-kb fragment containing exons 2 to 7. The CEL gene is located in a CNV region (http://dgv.tcag.ca/dgv/app/home). We observed duplications of this gene in DNA samples of several healthy individuals.
- **MODY 9** is caused by defects in the Paired box 4 gene (**PAX4**) on chromosome 7q32. This gene spans 5.4 kb of genomic DNA and consists of 9 exons.
- **MODY 10** has been linked to mutations in the insulin gene (**INS**) on chromosome 11p15. This gene has 2 exons and a size of 1.6 kb. Please note that INS gene mutations that cause MODY are all heterozygous missense mutations with a dominant-negative mode of action; a heterozygous deletion of the INS gene might not result in diabetes.
- **MODY 11** has been linked to defects in the B lymphoid tyrosine kinase gene (**BLK**). BLK has 13 exons and spans about 71 kb of genomic DNA. No probes for this gene are included in P241 or P357.

Data analysis

The P357-A3 MODY Mix 2 probemix contains 45 MLPA probes with amplification products between 131 and 490 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, 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.

Data generated by this probemix can first be normalised intra-sample by dividing the peak height of each probe's amplification product by the total peak height of only the reference probes in this 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 no changes occurred in the genomic regions recognised by the reference probes.
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 P357-A3 MODY Mix 2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position				
		reference	HNF1B	PAX4/PDX1	KLF11/NEUROD1	CEL/INS
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					
131 *	Reference probe 16316-L22397	3q21				
136	**HNF1B** probe 09858-L12290		**Exon 7**			
142	**PAX4** probe 15491-L17346			**Exon 1**		
148 «	**KLF11** probe 15492-L17347				**Exon 2**	
154	Reference probe 10195-L10655	16q21				
160	**HNF1B** probe 07699-L12885		**Exon 2**			
165	**PAX4** probe 15493-L17348			**Exon 8**		
178	Reference probe 05458-L04861	22q11				
184	**PAX4** probe 15495-L17350			**Exon 3**		
190 «	**KLF11** probe 15496-L17351				**Exon 4**	
196	Reference probe 09102-L09161	4q25				
202	**HNF1B** probe 08298-L18620		**Exon 8**			
208	**INS** probe 15497-L17352					**Exon 2**
214 ~ «	**CEL** probe 15498-L17353					**Exon 2**
220	**PDX1** probe 15499-L17354			**Exon 2**		
232	**INS** probe 15500-L17355					**Exon 3**
238	**HNF1B** probe 07700-L07459		**Exon 3**			
244	**INS** probe 15501-L17356					**Exon 1**
250 «	**KLF11** probe 15502-L17357				**Exon 3**	
264 ~ «	**CEL** probe 15503-L17358					**Exon 7**
274	**HNF1B** probe 07702-L07461		**Exon 4**			
283	Reference probe 04404-L02610	14q22				
292	**PAX4** probe 15504-L17359			**Exon 5**		
301	**HNF1B** probe 08299-L09335		**Exon 9**			
309 ~ «	**CEL** probe 15505-L17360					**Exon 4**
317	**NEUROD1** probe 15506-L17361				**Exon 2**	
326 ~ «	**CEL** probe 15507-L18618					**Exon 6**
335	**HNF1B** probe 07698-L18619		**Exon 1**			
346	Reference probe 10664-L11246	6p12				
355	**PDX1** probe 15508-L17363			**Exon 1**		
364	**HNF1B** probe 07701-L07460		**Exon 4**			
373	**PAX4** probe 15509-L17364			**Exon 6**		
382	**HNF1B** probe 11551-L12298		**Exon 5**			
391	Reference probe 12522-L13572	18q21				
400 ~ «	**CEL** probe 15510-L17365					**Exon 5**
409	**PAX4** probe 15511-L17366			**Exon 2**		
417	**NEUROD1** probe 15512-L17367				**Exon 1**	
427	Reference probe 08787-L11328	10q21				
436	**PAX4** probe 15513-L17368			**Exon 7**		
445 «	**KLF11** probe 15514-L17369				**Exon 1**	
454	**PAX4** probe 15515-L17370			**Exon 4**		
463	**HNF1B** probe 07704-L07463		**Exon 6**			
472	Reference probe 14846-L16554	3q12				
481	**PAX4** probe 15516-L17371			**Exon 9**		
490	Reference probe 13814-L15308	5q14				

* New in version A3 (from lot A3-1217 onwards).
« 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.
~ CEL is located in a CNV region (http://dgv.tcag.ca/dgv/app/home). We observed duplications of this gene in several DNA samples from healthy individuals.

Note: Exon numbering used here may differ from 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. P357 probes arranged according to chromosomal location

Table 2a. KLF11, 2p25.1

Length (nt)	SALSA MLPA probe	KLF11 exon	Ligation site NM_003597.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*163-165 (ex 1)*		
445 «	15514-L17369	Exon 1	178-179	ACACGCCGGACT-TCGCAGGCCCAG	2.5 kb
148 «	15492-L17347	Exon 2	281-282	TTGCAGCATCTT-GGAGCAGACAGA	2.0 kb
250 «	15502-L17357	Exon 3	1058-1059	AAGTAGCATGTT-ACCAGCTTTTTT	4.1 kb
190 «	15496-L17351	Exon 4	1548-1547, reverse	CTGCGCATGAAA-CGTCGGTCACAC	
		stop codon	*1699-1701 (ex 4)*		

The NM_003597.4 sequence represents transcript variant 1, a reference standard in the NCBI RefSeqGene project.

Table 2b. NEUROD1, 2q31.3

Length (nt)	SALSA MLPA probe	NEUROD1 exon	Ligation site NM_002500.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*248-250 (ex 2)*		
417	15512-L17367	Exon 1	226-227	TCAGGACCTACT-AACAACAAAGGT	2.1 kb
317	15506-L17361	Exon 2	745-744, reverse	TGAACGAAGGAG-ACCAGGTCTGGG	
		stop codon	*1316-1318 (ex 2)*		

The NM_002500.3 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2c. PAX4, 7q32.1

Length (nt)	SALSA MLPA probe	PAX4 exon	Ligation site NM_006193.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*207-209 (ex 1)*		
142	15491-L17346	Exon 1	89-88, reverse	CCACCGAGTGCA-TCCTCTCCTGGG	0.6 kb
409	15511-L17366	Exon 2	382-383	CACAGGTGTCTT-GGAGCCAAAGGG	0.5 kb
184	15495-L17350	Exon 3	580-579, reverse	GTCCCTGGTCCT-CCTGTAATGCCC	0.6 kb
454	15515-L17370	Exon 4	30 nt before exon 4	TCACCATCACAA-ACCACCACAAAC	0.4 kb
292	15504-L17359	Exon 5	1 nt before exon 5	TCGATCTCCGCA-GAGTTCCAGCGT	0.5 kb
373	15509-L17364	Exon 6	878-877, reverse	TGCATTTCCCAC-TTGAGCTTCTCT	1.1 kb
436	15513-L17368	Exon 7	944-943, reverse	ACCTGTGCAGAG-ATGATTCCTGGG	0.4 kb
165	15493-L17348	Exon 8	1054-1055	AGAAAGGTGTCT-GAGTGACACCCC	0.6 kb
481	15516-L17371	Exon 9	1298-1297, reverse	GGCAGGACGGTA-AGGACAATGGGC	
		stop codon	*1236-1238 (ex 9)*		

The NM_006193.2 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2d. CEL, 9q34.2

Length (nt)	SALSA MLPA probe	CEL exon	Ligation site NM_001807.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*17-19 (ex 1)*		
214 «	15498-L17353	Exon 2	135-136	GGAAGGCGTCAA-TAAGAAGCTCGG	0.7 kb
309 «	15505-L17360	Exon 4	460-461	AACTACCTGTAT-GACGGCGAGGAG	1.5 kb
400 «	15510-L17365	Exon 5	648-647, reverse	CGAAGAGCGTGA-TGTTGTTGGGGT	0.3 kb
326 «	15507-L18618	Exon 6	735-736	CCGGCGAGCCAT-CAGCCAGAGCGG	0.2 kb
264 «	15503-L17358	Exon 7	826-827	GTGGGTTGCCCT-GTGGGTGATGCC	
		stop codon	*2285-2287 (ex 11)*		

The NM_001807.4 sequence is a reference standard in the NCBI RefSeqGene project.

The CEL gene has 11 exons. Due to sequence similarity with the CEL pseudogene CELP, only probes for exons 2 to 7 of CEL are included. Compared to CEL, CELP lacks a 4.8-kb fragment containing exons 2 to 7. The CEL gene is located in a CNV region (http://dgv.tcag.ca/dgv/app/home). We observed duplications of this gene in several samples.

Table 2e. INS, 11p15.5

Length (nt)	SALSA MLPA probe	INS exon	Ligation site NM_000207.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*60-62 (ex 2)*		
244	15501-L17356	Exon 1	37-38	AAGAGGCCATCA-AGCAGGTCTGTT	0.4 kb
208	15497-L17352	Exon 2	233-232, reverse	TGCAGGTCCTCT-GCCTCCCGGCGG	1.0 kb
232	15500-L17355	Exon 3	10 nt after exon 3	GCCCTGCTGTGC-CGTCTGTGTGTC	
		stop codon	*390-392 (ex 3)*		

The NM_000207.2 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Table 2f. PDX1, 13q12.2

Length (nt)	SALSA MLPA probe	PDX1 exon	Ligation site NM_000209.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*109-111 (ex 1)*		
355	15508-L17363	Exon 1	159-160	TACAAGGACCCA-TGCGCGTTCCAG	5.8 kb
220	15499-L17354	Exon 2	2200-2201	TGTTCCGAGGTA-GAGGCTGTGCTG	
		stop codon	*958-960 (ex 2)*		

The NM_000209.3 sequence is a reference standard in the NCBI RefSeqGene project.

Table 2g. HNF1B, 17q12

Length (nt)	SALSA MLPA probe	HNF1B Exon	Ligation site NM_000458.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*195-197 (ex 1)*		
335	07698-L18619	Exon 1	199-200	TTGGAAAATGGT-GTCCAAGCTCAC	5.3 kb
160	07699-L12885	Exon 2	591-592	TGCAGCAACACA-ACATCCCCCAGA	5.8 kb
238	07700-L07459	Exon 3	787-789	AGACAAAAGCAG-TCAGGATCAGCT	2.0 kb
364	07701-L07460	Exon 4	1070-1069, reverse	ACACGGACCTCA-GTGACCAAGTTG	0.3 kb
274	07702-L07461	Exon 4	73 nt after exon 4	CTTATCTGGTTT-AAGGGTTTTCAG	20.8 kb
382	11551-L12298	Exon 5	6 nt before exon 5	GTTTGTGTTGTT-TTGCAGGAGTGC	5.6 kb
463	07704-L07463	Exon 6	1418-1419	GTCTCAGGAGGA-GGTTTGCCCCCA	4.0 kb
136	09858-L12290	Exon 7	1653-1652, reverse	GCTCTGCTGCAT-GAGGGGCTGCTG	2.0 kb
202	08298-L18620	Exon 8	1817-1818	AGCAGCATCAGT-ACACTCACCAAC	12.0 kb
301	08299-L09335	Exon 9	2098-2099	CTCTCCCACGAT-GTCAAGGACTCC	
		stop codon	*1866-1868 (ex 9)*		

The NM_000458.3 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

« 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.

Note: Exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P357-A3 MODY Mix 2 sample picture

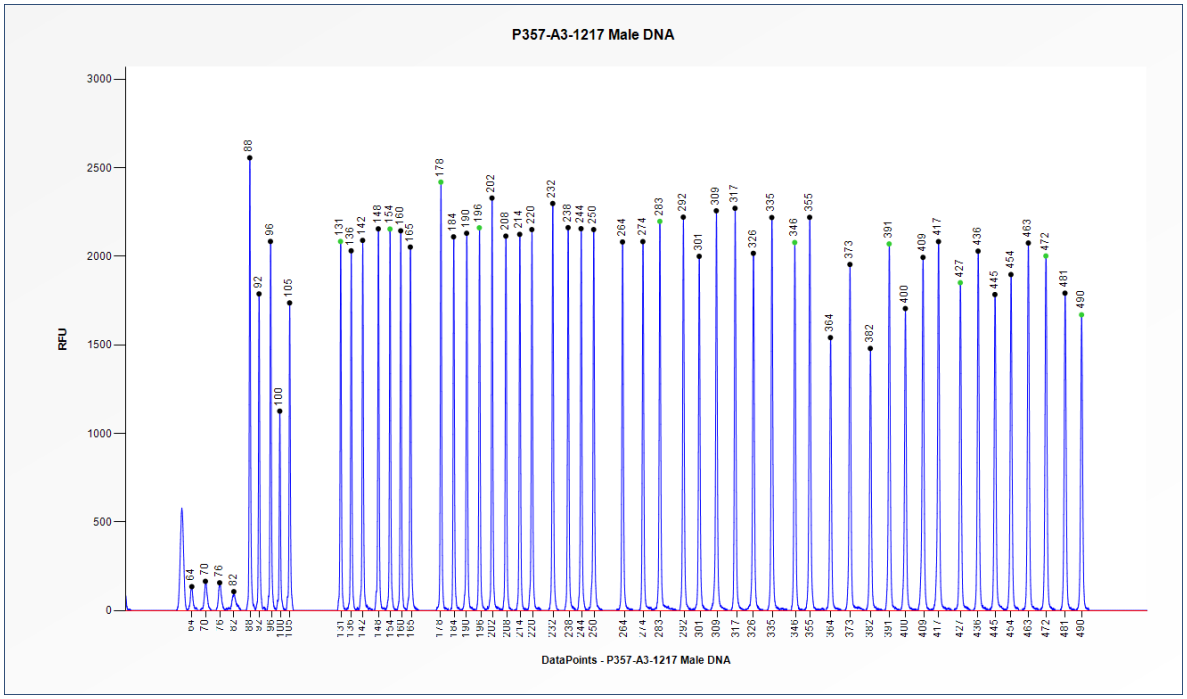

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA® MLPA® probemix P357-A3 MODY Mix 2 (lot A3-1217).

Implemented Changes – compared to the previous product description versions.
Version 07 – 13 February 2018 (55) - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). - ME033 added to related probemixes. - Name changed from P357 MODY mix-2 to P357 MODY Mix 2. *Version 06 – 13 April 2017 (55)* - Warnings added in Table 1 and 2, 190 nt probe 15496-L17351, 214 nt probe 15498-L17353, 264 nt probe 15503-L17358, 309 nt probe 15505-L17360, 326 nt probe 15507-L18618, and 400 nt probe 15510-L17365. - Minor textual changes. *Version 05 – 12 August 2015 (54)* - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). - Ligation sites of the NEUROD1 and HNF1B probes adjusted. - Exon numbering of the KLF11 and INS probes changed. *Version 04 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 03 (48)* - Remark on RefSeqGene standard and transcript variant added below Table 2. - Small correction of chromosomal locations in Table 1. - Ligation sites of the probes targeting the NEUROD1 gene updated according to new version of the NM_reference sequence. - Various minor textual and layout changes. *Version 02 (46)* - Note added under table 1 for the HNF1B probes. *Version 01 (46)* - Not applicable; new document