Product Description

SALSA® MLPA® Probemix P357-A3 MODY Mix 2

To be used with the MLPA General Protocol.

Version A3
For complete product history see page 10.

Catalogue numbers:

- **P357-025R:** SALSA MLPA Probemix P357 MODY Mix 2, 25 reactions.
- **P357-050R:** SALSA MLPA Probemix P357 MODY Mix 2, 50 reactions.
- **P357-100R:** SALSA MLPA Probemix P357 MODY Mix 2, 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.mrcholland.com).

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

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 P357 MODY Mix 2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *KLF11*, *NEUROD1*, *PAX4*, *CEL*, *INS*, *PDX1* and *HNF1B* genes, which are associated with Maturity-Onset Diabetes of the Young.

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 an autosomal dominant 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 below, 14 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. 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.

The MODY genes

- **MODY 1** is a result of defects in the Hepatocyte nuclear factor-4-alpha gene (***HNF4A***) 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 chromosome 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***). The *PDX1* gene (two exons) spans ~6 kb of genomic DNA and is located on chromosome 13q12.2, ~27 Mb from the p-telomere.

- **MODY 5** have been associated with the HNF1 Homeobox B gene (***HNF1B***). The *HNF1B* gene (nine exons) spans ~59 kb of genomic DNA and is located on chromosome 17q12, ~33 Mb from the p-telomere. 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 neuronal differentiation 1 gene (***NEUROD1***). The *NEUROD1* gene (two exons) spans ~4 kb of genomic DNA and is located on chromosome 2q31.3, ~182 Mb from the p-telomere.
- **MODY 7** is caused by mutations in the Krüppel-Like Factor 11 gene (***KLF11***) on chromosome 2p25.1. The *KLF11* gene (four exons) spans ~11 kb of genomic DNA and is located on chromosome 2p25.1, ~10 Mb from the p-telomere.
- **MODY 8** has been associated with defects in Carboxyl-ester lipase gene (***CEL***). The *CEL* gene (11 exons) spans ~10 kb of genomic DNA and is located on chromosome 9q34.2, ~135 Mb from the p-telomere. 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***). The *PAX4* gene (12 exons) spans ~8 kb of genomic DNA and is located on chromosome 7q32.1, ~127 Mb from the p-telomere.
- **MODY 10** has been linked to mutations in the insulin gene (***INS***). The *INS* gene (three exons) spans ~1.4 kb of genomic DNA and is located on chromosome 11p15.5, ~138 Mb from the p-telomere. 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. The ME033 probemix contains *INS* probes that target the same sequences as the *INS* probes in this P357 probemix.
- **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 on chromosome 8p23.1. No probes for this gene are included in P241 or P357.
- **MODY 12** has been linked to defects in the ATP binding cassette subfamily C member 8 gene (***ABCC8***). The *ABCC8* gene consists of 39 exons and spans 84 kb of genomic DNA on chromosome 11p15.1. Probes for this gene are included in the P117 probemix.
- **MODY 13** has been linked to defects in the potassium inwardly rectifying channel subfamily J member 11 gene (***KCNJ11***). The *KCNJ11* gene consists of one exon and spans 3.4 kb of genomic DNA on chromosome 11p15.1. Probes for this gene are included in the ME033 probemix.
- **MODY 14** has been linked to defects in the gene encoding adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (***APPL1***). The *APPL1* gene consists of 22 exons and spans 46 kb of genomic DNA on chromosome 3p14.3. No probes for this gene are included in P241 or P357.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK500456/.

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 *KLF11*, *NEUROD1*, *PAX4*, *CEL*, *INS*, *PDX1* and *HNF1B* exon numbering used in this P357-A3 MODY Mix 2 product description is the exon numbering from the NG_017199.1, LRG_1119, NG_012848.1, NG_016394.1, NG_007114.1, NG_008183.1 and NG_013019.2 sequences, respectively. The *PAX4* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2c. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG or LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content

The SALSA MLPA Probemix P357-A3 MODY Mix 2 contains 45 MLPA probes with amplification products between 131 and 490 nucleotides (nt). This includes four probes for the *KLF11* gene (one for each exon), two probes for the *NEUROD1* gene (one for each exon), nine probes for the *PAX4* gene (for exons 4 to 12), five probes for the *CEL* gene, three probes for the *INS* gene (one for each exon), two probes for the *PDX1* gene (one for each exon) and ten probes for the *HNF1B* gene (one for each exon and two for exon 4). In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.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.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.com).

MLPA technique validation

Internal validation of the MLPA technique using 16 DNA samples from healthy individuals 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 (except the *CEL* 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 different unrelated individuals who are from families without a history of diabetes. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers from the Coriell Institute in the table below have been tested with this P357-A3 probemix at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Altered target genes in P357-A3	Expected copy number alteration
NA00945	*KLF11*	Heterozygous deletion
NA01353	*KLF11*	Heterozygous duplication
NA04409	*KLF11*	Heterozygous duplication
NA10401	*KLF11*, *NEUROD1*	Heterozygous duplication
NA03918	*NEUROD1*	Heterozygous deletion
NA12519	*PAX4*	Homozygous duplication
NA13685	*CEL*	Heterozygous duplication
NA03330	*PDX1*	Heterozygous duplication
NA12606	*PDX1*	Heterozygous duplication
NA20359	*HNF1B*	Heterozygous duplication

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.mrcholland.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 final ratio (FR) of each individual probe (except for the *CEL* probes) in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *KLF11* or *CEL* gene. 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.
- 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: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P357 specific notes:

- *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.
- *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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in MODY genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P357 MODY Mix 2.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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.

***KLF11*, *NEUROD1*, *PAX4*, *CEL*, *INS*, *PDX1* and *HNF1B* mutation databases**

https://databases.lovd.nl/shared/genes/KLF11
https://databases.lovd.nl/shared/genes/NEUROD1
https://databases.lovd.nl/shared/genes/PAX4
https://databases.lovd.nl/shared/genes/CEL
https://databases.lovd.nl/shared/genes/INS

https://databases.lovd.nl/shared/genes/PDX1
https://databases.lovd.nl/shared/genes/HNF1B
We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 SNVs and unusual results (e.g., a duplication of *INS* exons 1 and 3 but not exon 2) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P357-A3 MODY Mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*HNF1B*	*PAX4/PDX1*	*KLF11/NEUROD1*	*CEL/INS*
64-105	Control fragments – see table in probemix content section for more information					
131 *	Reference probe 16316-L22397	3q				
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	16q				
160 »	**HNF1B probe** 07699-L12885		**Exon 2**			
165	**PAX4 probe** 15493-L17348			**Exon 8**		
178	Reference probe 05458-L04861	22q				
184	**PAX4 probe** 15495-L17350			**Exon 3**		
190 «	**KLF11 probe** 15496-L17351				**Exon 4**	
196	Reference probe 09102-L09161	4q				
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	14q				
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	6p				
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	18q				
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	10q				
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	3q				
481	**PAX4 probe** 15516-L17371			**Exon 9**		
490	Reference probe 13814-L15308	5q				

[a] See section Exon numbering on page 2 for more information.

* New in version A3.
« 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.

» The *INS* probes in this P357 probemix detect the same sequences as the *INS* probes in SALSA MLPA Probemix ME033. The indicated *HNF1B* probes in this probemix detect the same sequences as the *HNF1B* probes in SALSA MLPA Probemix P241.
~ *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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P357-A3 probes arranged according to chromosomal location

Table 2a. *KLF11* gene, 2p

Length (nt)	SALSA MLPA probe	*KLF11* exon[a]	Ligation site NM_003597.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*168-170 (Exon 1)*		
445 «	15514-L17369	Exon 1	183-184	ACACGCCGGACT-TCGCAGGCCCAG	2.5 kb
148 «	15492-L17347	Exon 2	286-287	TTGCAGCATCTT-GGAGCAGACAGA	2.0 kb
250 «	15502-L17357	Exon 3	1063-1064	AAGTAGCATGTT-ACCAGCTTTTTT	4.1 kb
190 «	15496-L17351	Exon 4	1553-1552 *reverse*	CTGCGCATGAAA-CGTCGGTCACAC	
		stop codon	*1704-1706 (Exon 4)*		

Table 2b. *NEUROD1* gene, 2q

Length (nt)	SALSA MLPA probe	*NEUROD1* exon[a]	Ligation site NM_002500.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*100-102 (Exon 2)*		
417	15512-L17367	Exon 1	78-79	TCAGGACCTACT-AACAACAAAGGT	2.1 kb
317	15506-L17361	Exon 2	597-596 *reverse*	TGAACGAAGGAG-ACCAGGTCTGGG	
		stop codon	*1168-1170 (Exon 2)*		

Table 2c. *PAX4* gene, 7q

Length (nt)	SALSA MLPA probe	*PAX4* exon[a]	Ligation site NM_001366110.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*407-409 (Exon 3)*		
142	15491-L17346	Exon 4 (1)	107 nt before exon 4 *reverse*	CCACCGAGTGCA-TCCTCTCCTGGG	0.6 kb
409	15511-L17366	Exon 5 (2)	606-607	CACAGGTGTCTT-GGAGCCAAAGGG	0.5 kb
184	15495-L17350	Exon 6 (3)	804-803 *reverse*	GTCCCTGGTCCT-CCTGTAATGCCC	0.6 kb
454	15515-L17370	Exon 7 (4)	30 nt before exon 7	TCACCATCACAA-ACCACCACAAAC	0.4 kb
292	15504-L17359	Exon 8 (5)	1 nt before exon 8	TCGATCTCCGCA-GAGTTCCAGCGT	0.5 kb
373	15509-L17364	Exon 9 (6)	1102-1101 *reverse*	TGCATTTCCCAC-TTGAGCTTCTCT	1.1 kb
436	15513-L17368	Exon 10 (7)	1168-1167 *reverse*	ACCTGTGCAGAG-ATGATTCCTGGG	0.4 kb
165	15493-L17348	Exon 11 (8)	1278-1279	AGAAAGGTGTCT-GAGTGACACCCC	0.6 kb
481	15516-L17371	Exon 12 (9)	1522-1521 *reverse*	GGCAGGACGGTA-AGGACAATGGGC	
		stop codon	*1460-1462 (Exon 12)*		

Table 2d. *CEL* gene, 9q

Length (nt)	SALSA MLPA probe	*CEL* exon[a]	Ligation site NM_001807.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*23-25 (Exon 1)*		
214 « ~	15498-L17353	Exon 2	132-133	GGAAGGCGTCAA-TAAGAAGCTCGG	0.7 kb
309 « ~	15505-L17360	Exon 4	457-458	AACTACCTGTAT-GACGGCGAGGAG	1.5 kb
400 « ~	15510-L17365	Exon 5	645-644 *reverse*	CGAAGAGCGTGA-TGTTGTTGGGGT	0.3 kb
326 « ~	15507-L18618	Exon 6	732-733	CCGGCGAGCCAT-CAGCCAGAGCGG	0.2 kb
264 « ~	15503-L17358	Exon 7	823-824	GTGGGTTGCCCT-GTGGGTGATGCC	
		stop codon	*2282-2284 (Exon 11)*		

Table 2e. *INS* gene, 11p

Length (nt)	SALSA MLPA probe	*INS* exon[a]	Ligation site NM_000207.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*60-62 (Exon 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 (Exon 3)*		

Table 2f. *PDX1* gene, 13q

Length (nt)	SALSA MLPA probe	*PDX1* exon[a]	Ligation site NM_000209.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*140-142 (Exon 1)*		
355	15508-L17363	Exon 1	190-191	TACAAGGACCCA-TGCGCGTTCCAG	5.7 kb
220	15499-L17354	Exon 2	2231-2232	TGTTCCGAGGTA-GAGGCTGTGCTG	
		stop codon	*989-991 (Exon 2)*		

Table 2g. *HNF1B* gene, 17q

Length (nt)	SALSA MLPA probe	*HNF1B* exon[a]	Ligation site NM_000458.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*176-178 (Exon 1)*		
335 »	07698-L18619	Exon 1	180-181	TTGGAAAATGGT-GTCCAAGCTCAC	5.3 kb
160 »	07699-L12885	Exon 2	572-573	TGCAGCAACACA-ACATCCCCCAGA	5.8 kb
238	07700-L07459	Exon 3	768-769	AGACAAAAGCAG-TCAGGATCAGCT	2.0 kb
364 » #	07701-L07460	Exon 4	1051-1050 *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	5.7 kb
463 »	07704-L07463	Exon 6	1399-1400	GTCTCAGGAGGA-GGTTTGCCCCCA	4.0 kb
136 »	09858-L12290	Exon 7	1634-1633 *reverse*	GCTCTGCTGCAT-GAGGGGCTGCTG	2.0 kb
202 »	08298-L18620	Exon 8	1798-1799	AGCAGCATCAGT-ACACTCACCAAC	12.0 kb
301	08299-L09335	Exon 9	2079-2080	CTCTCCCACGAT-GTCAAGGACTCC	
		stop codon	*1847-1849 (Exon 9)*		

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« 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.
» The *INS* probes in this P357 probemix detect the same sequences as the *INS* probes in SALSA MLPA Probemix ME033. The indicated *HNF1B* probes in this probemix detect the same sequences as the *HNF1B* probes in SALSA MLPA Probemix P241.
~ *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.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

- ME033 TNDM: Contains probes for several genes involved in transient neonatal diabetes mellitus, including the *INS* probes that target the same sequences as the *INS* probes in this probemix (MODY 10) and *KCNJ11* probes (MODY 13).

- P117 ABCC8: Contains probes for the *ABCC8* gene, involved in familial hyperinsulinemic hypoglycemia 1 and linked to MODY 12.
- P241 MODY Mix 1: Contains probes for *HNF4A*, *GCK*, *HNF1A* and *HNF1B* genes, involved in MODY 1, 2, 3 and 5, respectively.

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.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P357 MODY Mix 2

- Horikawa Y et al. (2018). NEUROD1-deficient diabetes (MODY6): Identification of the first cases in Japanese and the clinical features. Pediatr Diabetes. 19:236-242.
- Morisada N et al. (2019). Clinical characteristics of HNF1B-related disorders in a Japanese population. Clin Exp Nephrol. 23:1119-1129.

P357 product history	
Version	*Modification*
A3	One reference probe has been replaced.
A2	Two reference probes and the 88 and 96 nt control fragments have been replaced (QDX2).
A1	First release.

Implemented changes in the product description

Version A3-01 – 28 July 2021 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting all genes updated according to new versions of the NM_ reference sequences.
- Exon numbering of the *PAX4* gene has been changed.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

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.

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