Product Description SALSA® MLPA® Probemix P222-B2 LCA mix-2

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, two reference probes have been replaced. For complete product history see page 7.

Catalogue numbers:
- **P222-025R:** SALSA MLPA Probemix P222 LCA mix-2, 25 reactions.
- **P222-050R:** SALSA MLPA Probemix P222 LCA mix-2, 50 reactions.
- **P222-100R:** SALSA MLPA Probemix P222 LCA 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.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 P222 LCA mix-2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GUCY2D*, *RDH12* and *RPGRIP1* genes, which are associated with Leber Congenital Amaurosis (LCA). This probemix can also be used to detect the presence of the c.2991+1655A>G (rs281865192; p.Cys998X) point mutation in *CEP290*, which is also linked to LCA.

LCA comprises of a group of early-onset childhood retinal dystrophies and is characterised by vision loss, nystagmus and severe retinal dysfunction. It is the most common inherited cause of blindness. Pathogenic variants in at least seventeen genes are known to cause LCA. This probemix contains probes for four of these genes.

The protein encoded by the *GUCY2D* gene is retina-specific guanylate cyclase, located in the photoreceptor cells where it is involved in phototransduction. Defects in the *GUCY2D* gene account for 6-21% of all LCA cases. The protein encoded by the *RDH12* gene is an NADPH-dependent retinal reductase, which metabolizes both trans- and cis-retinols. Defects in the *RDH12* gene account for 4-10% of all LCA cases. The protein encoded by the *RPGRIP1* gene is retinitis pigmentosa GTPase regulator interacting protein, a key component of photoreceptor cells. Defects in the *RPGRIP1* gene account for ~5% of all LCA cases. The protein encoded by the *CEP290* gene is centrosomal protein of 290 kDa, involved in ciliary assembly and ciliary trafficking of the photoreceptor cells. Defects in the *CEP290* gene account for 15%-22% of all LCA cases. The intronic mutation c.2991+1655A>G is the most common *CEP290* mutation associated with LCA and may account for up to 21% of LCA cases (den Hollander et al. 2006).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK531510/.

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 *GUCY2D*, *RDH12*, *RPGRIP1*, and *CEP290* exon numbering used in this P222-B2 LCA mix-2 product description is the exon numbering from the RefSeq transcripts NM_000180.4, NM_152443.3, NM_020366.3 and NM_025114.3, which are identical to the NG_009092.1, NG_008321.1, NG_008933.1 and LRG_694 sequences, respectively. The exon numbering and NM_ sequences used have been retrieved on 01/2020. 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 P222-B2 LCA mix-2 contains 44 MLPA probes with amplification products between 130 and 454 nucleotides (nt). This includes 18 probes for the *RPGRIP1* gene, one probe for each exon with the exception of exons 5, 7, 9, 12, 15 and 17, seven probes for the *RDH12* gene, one probe for each exon with the exception of exons 1 and 2, and eight probes for the *GUCY2D* gene, one probe each for exons 2, 5, 7, 10, 12, 14, 17 and 20. Furthermore, this probemix also contains one probe specific for the c.2991+1655A>G mutation in the *CEP290* gene, which will only generate a signal when the wildtype allele is present. A reduced signal can point towards the presence of the mutation **or** a deletion in intron 26. To facilitate discrimination between presence of the mutation or presence of a deletion, one additional probe for intron 26 of *CEP290* is included in the probemix. This probe is located at short distance from the wild-type probe. In case of a deletion, both probes are expected so show a reduced signal. In case the mutation is present, only the probe specific for the the c.2991+1655A>G mutation is expected to show a reduced signal. In addition to the above listed probes, nine 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.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, 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 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 Leber Congenital Amaurosis. 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. 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 for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous 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 *GUCY2D*, *RDH12*, *RPGRIP1* and *CEP290* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P222 LCA mix-2. The exception is the c.2991+1655A>G mutation in *CEP290*, for which a probe is included in this probemix.

- 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.

LOVD 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 *RDH12* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P222-B2 LCA mix-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*GUCY2D*	*RDH12*	*RPGRIP1*	*CEP290*
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 05169-L04550	8q24				
136	Reference probe 00662-L00158	6p21				
139	**RPGRIP1 probe** 07023-L06634				**Exon 10**	
143	**RDH12 probe** 08890-L09782			**Exon 5**		
148	**RPGRIP1 probe** 07018-L06629				**Exon 2**	
154	**GUCY2D probe** 07038-L06649		**Exon 7**			
161	Reference probe 14745-L18792	9q32				
172	**RPGRIP1 probe** 07032-L06643				**Exon 22**	
177	**RPGRIP1 probe** 07021-L06632				**Exon 6**	
184	**RPGRIP1 probe** 07024-L06635				**Exon 11**	
190	Reference probe 16424-L26299	18q21				
196	**RPGRIP1 probe** 07033-L08019				**Exon 23**	
202	**GUCY2D probe** 07035-L06646		**Exon 2**			
208	**RPGRIP1 probe** 07019-L06630				**Exon 3**	
214	Reference probe 06733-L28502	22q13				
220	**RPGRIP1 probe** 07029-L06640				**Exon 19**	
230	**RDH12 probe** 08889-L08851			**Exon 4**		
238	**RPGRIP1 probe** 07030-L06641				**Exon 20**	
247	**GUCY2D probe** 07037-L06648		**Exon 5**			
256	**GUCY2D probe** 07039-L08018		**Exon 10**			
262	**RPGRIP1 probe** 07031-L29169				**Exon 21**	
268 *	Reference probe 19040-L09299	3q29				
274 ∞	**CEP290 probe** 08887-L08849					**Intron 26**
283	**GUCY2D probe** 07041-L06652		**Exon 14**			
292	**RPGRIP1 probe** 07017-L06628				**Exon 1**	
301	**RPGRIP1 probe** 07022-L06633				**Exon 8**	
310	**RPGRIP1 probe** 07028-L06639				**Exon 18**	
319	**RDH12 probe** 08892-L08854			**Exon 7**		
328	**RPGRIP1 probe** 07020-L06631				**Exon 4**	
337	**GUCY2D probe** 07042-L06653		**Exon 17**			
346	**RPGRIP1 probe** 07027-L06638				**Exon 16**	
355	**RPGRIP1 probe** 07034-L06645				**Exon 24**	
364	**RDH12 probe** 08893-L08855			**Exon 8**		
371	**RPGRIP1 probe** 08141-L29174				**Exon 14**	
377	Reference probe 19135-L29175	21q21				
383	**GUCY2D probe** 07043-L06654		**Exon 20**			
391	**RDH12 probe** 08891-L08853			**Exon 6**		
400 ∞	**CEP290 probe** 08886-L08848					**Intron 26**
408	**RDH12 probe** 08894-L08856			**Exon 9**		
418	**RDH12 probe** 08888-L08850			**Exon 3**		
427	Reference probe 00680-L00121	7q34				
434 ±	**RPGRIP1 probe** 20916-L06636				**Exon 13**	
445	**GUCY2D probe** 20915-L06651		**Exon 12**			
454 *	Reference probe 06194-L22769	20q13				

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

* New in version B2.
∞ Wild type sequence detected. A lowered probe signal for the 400 nt probe can be due to a deletion in intron 26 or due to the presence of the c.2991+1655A>G mutation. In case of a deletion both the 400 nt and 274 nt probes are expected to show a lowered probe signal, whereas only the 400 nt probe will show a lowered probe signal if the mutation is present. Please note that other variants near the ligation site of the 400 nt probe can also cause a lowered signal. A positive result must be confirmed by another method.
± SNP rs147586703 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 2. P222-B2 probes arranged according to chromosomal location

Table 2a. *GUCY2D*

Length (nt)	SALSA MLPA probe	***GUCY2D*** **exon[a]**	Ligation site NM_000180.4	**Partial sequence[b] (24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*130-132 (Exon 2)*		
202	07035-L06646	Exon 2	13 nt before exon 2, 22 nt before ATG	CAGCCTGCTCCG-TCTGTGTTCGCA	4.0 kb
247	07037-L06648	Exon 5	9 nt before exon 5	CTCCTGTCACTG-TCCCTTCAGGAC	0.9 kb
154	07038-L06649	Exon 7	2 nt before exon 7, reverse	CCTGGGCCACCT-AGCAGGTAGAGG	4.6 kb
256	07039-L08018	Exon 10	2158-2159	TAGTGGATGGCA-GATTCGTACTCA	1.4 kb
445	20915-L06651	Exon 12	2466-2467	GCACCTGTCGAG-TGTATCCTCCTG	0.9 kb
283	07041-L06652	Exon 14	2743-2744	GGACACCAGTGG-AGCCCGAGTACT	1.1 kb
337	07042-L06653	Exon 17	3209-3210	CACTGTGGGGAT-TCTCCGTGCTCT	4.2 kb
383	07043-L06654	Exon 20	3538-3539	TGGGGTGAACTG-CTCCTTGGCAGG	
		stop codon	*3439-3441 (Exon 19)*		

Table 2b. *RDH12*

Length (nt)	SALSA MLPA probe	***RDH12*** **exon[a]**	Ligation site NM_152443.3	**Partial sequence[b] (24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*325-327 (Exon 3)*		
418	08888-L08850	Exon 3	255-256	GCCAGACCAGGA-ACCTGAGCCAGA	1.9 kb
230	08889-L08851	Exon 4	420-421	GGAGTGTGTAGA-ACAAATGTGCAG	0.7 kb
143	08890-L09782	Exon 5	581-582	AATCCGAGTGGA-TACAAAGAACTC	0.8 kb
391	08891-L08853	Exon 6	80 nt before exon 6	TGAACAAAGGGA-AAGGGCAATTAT	1.0 kb
319	08892-L08854	Exon 7	29 nt before exon 7	GTCATTCCACTT-TCAATCTTCCCT	2.2 kb
364	08893-L08855	Exon 8	74 nt before exon 8	TCACTTGTGTAT-TTTGCTGCAGGA	5.1 kb
408	08894-L08856	Exon 9	1629-1630	GCTGAGGCAAGA-AGAGCACCATCA	
		stop codon	*1273-1275 (Exon 9)*		

Table 2c. *RPGRIP1*

Length (nt)	SALSA MLPA probe	***RPGRIP1*** **exon[a]**	Ligation site NM_020366.3	**Partial sequence[b] (24 nt adjacent to ligation site)**	Distance to next probe
		start codon	*1-3 (Exon 1)*		
292 #	07017-L06628	Exon 1	31-32	CTACATCAGGAG-ACTTGCCAGTTA	6.7 kb
148	07018-L06629	Exon 2	122-123	CTTGAGCAGGAT-GAACCGGGAGGA	6.3 kb
208	07019-L06630	Exon 3	3 nt before exon 3	TCTATTTGTCCA-CAGGCTGAGGAC	1.6 kb
328	07020-L06631	Exon 4	556-557	CAAATGAAAACA-GAGGTGAAGTAG	5.2 kb
177	07021-L06632	Exon 6	4 nt before exon 6	GCGCCTTTCTCT-GCAGAGCTTCCA	4.2 kb
301	07022-L06633	Exon 8	995-996	GCTCAGGGCAGA-GCTGAAGGAAGA	5.8 kb
139	07023-L06634	Exon 10	1178-1179	TGACAGCTCCAG-TCAGCCCCACTG	2.3 kb
184	07024-L06635	Exon 11	1349-1350	CAACATACTTCA-GAAGCATAAACA	1.9 kb
434 ±	20916-L06636	Exon 13	1751-1752	AAGCCATGACCT-TCCAACATCTGG	2.8 kb
371	08141-L29174	Exon 14	1977-1978	TATTCCTTCTAT-GACTTTGAAACC	1.1 kb
346	07027-L06638	Exon 16	2485-2486	TGTACCGCTTCT-TCACCTTTTCTG	2.5 kb
310	07028-L06639	Exon 18	2925-2926	GAGGTTCCCATT-GAAGCTGGCCAG	1.8 kb
220	07029-L06640	Exon 19	3131-3132	GAAGTTCTCAGA-GACTAACAGCTT	4.4 kb
238	07030-L06641	Exon 20	3273-3274	TCTGAAGTCAGT-GAAGCACAAACT	8.4 kb
262	07031-L29169	Exon 21	3379-3380	TCTCCCTGGCCT-TCTACCCAGAGG	2.1 kb
172	07032-L06643	Exon 22	3599-3600	GCTGAATGGACA-AGATCCTGATCA	3.0 kb
196	07033-L08019	Exon 23	3654-3655	CCTCTGGATGAA-GAAAAGAAAGAA	2.9 kb
355	07034-L06645	Exon 24	3783-3784	ACCCCAATAGGA-AGGCTGAAGGTT	
		stop codon	*3859-3861 (Exon 24)*		

Table 2d. *CEP290*

Length (nt)	SALSA MLPA probe	*CEP290* exon[a]	Ligation site NM_025114.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*217-219 (Exon 2)*		
400 ∞	08886-L08848	Intron 26	1655 nt after exon 26	TGTAATTGTGAA-TATCTCATACCT	0.1 kb
274 ∞	08887-L08849	Intron 26	1790 nt after exon 26, reverse	ACAGGGTAGGAT-TCATGTTTAGAA	
		stop codon	*7654-7656 (Exon 54)*		

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.

∞ Wild type sequence detected. A lowered probe signal for the 400 nt probe can be due to a deletion in intron 26 or due to the presence of the c.2991+1655A>G mutation. In case of a deletion both the 400 nt and 274 nt probes are expected to show a lowered probe signal, whereas only the 400 nt probe will show a lowered probe signal if the mutation is present. Please note that other variants near the ligation site of the 400 nt probe can also cause a lowered signal. A positive result must be confirmed by another method.
± SNP rs147586703 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
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.

Related SALSA MLPA probemixes

P221 LCA mix-1 Contains probes for the *AIPL1*, *CRB1*, *CRX*, *LCA5*, and *RPE65* genes, involved in Leber Congenital Amaurosis.

References

- den Hollander AI et al. (2006). Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. *Am J Hum Genet*. 79:556-561.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
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Selected publications using SALSA MLPA Probemix P222 LCA mix-2

- Han J et al. (2017). Diagnostic application of clinical exome sequencing in Leber congenital amaurosis. *Mol Vis*. 23:649-659.
- Hosono K et al. (2018). Molecular diagnosis of 34 Japanese families with Leber congenital amaurosis using targeted next generation sequencing. *Sci Rep*. 8:8279.
- Sanchez-Navarro I et al. (2018). Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. *Sci Rep*. 8:5285.

P222 Product history	
Version	*Modification*
B2	Two reference probes have been replaced.
B1	One target has been removed, five reference probes have been replaced, and three reference probes have been added.
A2	The 88, 96, 100 and 105 nt control fragments (QDX2) have been included.
A1	First release.

Implemented changes in the product description
Version B2-01 – 06 February 2020 (02P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *GUCY2D* gene updated according to new version of the NM_ reference sequence. - Version of the NM_ reference sequence updated for the *RDH12* and *CEP290* genes. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Warning added for the 434 nt probe (20916-L06636) in Table 1 and 2; probe signal may be affected by SNP rs147586703. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - Probe remark about incomplete sample DNA denaturation due to the presence of salt removed below Table 1 and 2 for the following probes: 202 nt probe 07035-L06646, 247 nt probe 07037-L06648, 154 nt probe 07038-L06649. *Version 12 – 11 March 2016 (55)* - 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). - Various minor textual changes. - Various minor layout changes. - "Peak area" replaced with "peak height". - Updated link for "Database of Genomic Variants". - Two warnings added to Table 1 and Table 2a: 202 nt GUCY2D probe 07035-L06646 and 247 nt GUCY2D probe 07037-L06648. - One warning removed from Table 1 and Table 2c: 195 nt RPGRIP1 probe 07033-L08019. - Manufacturer's address adjusted. *Version 11 (48)* - Electropherogram picture of the old buffer (introduced Dec. 2012) removed.

More information: www.mlpa.com; www.mlpa.eu	
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