Product Description
SALSA® MLPA® Probemix P221-C2 LCA mix-1

To be used with the MLPA General Protocol.

Version C2
For complete product history see page 8.

Catalogue numbers:
- **P221-025R:** SALSA MLPA Probemix P221 LCA mix-1, 25 reactions.
- **P221-050R:** SALSA MLPA Probemix P221 LCA mix-1, 50 reactions.
- **P221-100R:** SALSA MLPA Probemix P221 LCA mix-1, 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 P221 LCA mix-1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *AIPL1*, *CRB1*, *CRX*, *LCA5* and *RPE65* genes, which are associated with Leber congenital amaurosis syndrome.

Leber congenital amaurosis (LCA) syndrome comprises 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 seventeen genes are known to cause LCA, five of these genes are covered by this probemix.

The *AIPL1* gene encodes the aryl hydrocarbon receptor interacting protein-like 1, located in the retinal photoreceptor cells, where it may act as a molecular chaperone. Defects in the *AIPL1* gene account for ~4%-8% of all LCA cases. The protein encoded by the *CRB1* gene is crumbs family member 1, which plays a role in photoreceptor morphogenesis in the retina. Defects in this gene account for ~10% of all LCA cases. The *CRX* gene encodes the cone-rod homeobox protein, which regulates retinal photoreceptor cell-specific gene transcription and plays a role in the differentiation of photoreceptor cells. Defects in this gene account for ~3% of all LCA cases. Leber congenital amaurosis 5 protein is encoded by the *LCA5* gene, and is involved in centrosomal or ciliary functions. Approximately 1%-2% of all LCA cases are caused by defects in the *LCA5* gene. Lastly, the *RPE65* gene encodes the retinal pigment epithelium-specific protein 65kDa, which is located in the retinal pigment epithelium where it is involved in the production of 11-cis retinal and in visual pigment regeneration. Defects in *RPE65* account for 6%-16% of all LCA cases (Chacon-Camacho and Zenteno 2015).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1298/.

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 *AIPL1*, *CRB1*, *CRX*, *LCA5* and *RPE65* exon numbering used in this P221-C2 LCA mix-1 product description is the exon numbering from the NG_008474.1, NG_008483.2, NG_008605.1, NG_016011.1 and NG_008472.2 sequences. 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 LRG/NG 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 P221-C2 LCA mix-1 contains 54 MLPA probes with amplification products between 128 and 503 nucleotides (nt). This includes six probes for the *AIPL1* gene, nine probes for the *LCA5* gene, 13 probes for the *CRB1* gene, 14 probes for the *RPE65* gene and four probes for the *CRX* gene. In addition, eight reference probes are included and detect eight 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-fragments (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 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 Leber congenital amaurosis syndrome. 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 NA00214, NA10946, HG01802 and HG02397 from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control samples (see table below). The quality of cell lines can change, therefore samples should be validated before use.

Sample ID Coriell	Genotype	Probes affected	Expected FR
NA00214	Heterozygous deletion of *CRB1* gene	All CRB1 probes	0.5
NA10946	Heterozygous deletion of *LCA5* gene	All LCA5 probes	0.5
HG01802	Heterozygous duplication of *LCA5* gene	All LCA5 probes	1.5
HG02397	Heterozygous duplication of *CRX* gene	All CRX probes	1.5

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 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 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. 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *AIPL1*, *CRB1*, *CRX*, *LCA5* and *RPE65* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P221-C2 LCA mix-1.
- 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.

***RPE65*, *LCA5*, *AIPL1*, *CRB1*, *CRX* mutation databases**

https://databases.lovd.nl/shared/genes/RPE65; https://databases.lovd.nl/shared/genes/LCA5; https://databases.lovd.nl/shared/genes/AIPL1; https://databases.lovd.nl/shared/genes/CRB1; https://databases.lovd.nl/shared/genes/CRX. 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 SNVs and unusual results (e.g., a duplication of *RPE65* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P221-C2 LCA mix-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*AIPL1*	*CRB1*	*CRX*	*LCA5*	*RPE65*
64-105	Control fragments – see table in probemix content section for more information						
128	Reference probe 00797-L00093	5q					
131	**LCA5 probe** 10266-L20190					**Exon 1**	
137	**AIPL1 probe** 06972-L14818		**Exon 4**				
142	**CRB1 probe** 06967-L06547			**Exon 9**			
148	**RPE65 probe** 04320-L28624						**Exon 14**
153	**AIPL1 probe** 06974-L06554		**Exon 6**				
160	**CRX probe** 08771-L09336				**Exon 2**		
166	**RPE65 probe** 03599-L02966						**Exon 1**
172	**AIPL1 probe** 06973-L06553		**Exon 5**				
178	**RPE65 probe** 06953-L06533						**Exon 8**
184	Reference probe 08788-L08812	10q					
190	**CRB1 probe** 07366-L06545			**Exon 8**			
196	**RPE65 probe** 06954-L06534						**Exon 11**
202	**RPE65 probe** 03600-L02967						**Exon 3**
208	**CRB1 probe** 06957-L20126			**Exon 1**			
214	**RPE65 probe** 06956-L28625						**Exon 13**
220	**AIPL1 probe** 06971-L06551		**Exon 3**				
226	**LCA5 probe** 10267-L10779					**Exon 2**	
233	Reference probe 19624-L26861	10p					
238	**RPE65 probe** 20555-L14777						**Exon 4**
245	**LCA5 probe** 20562-L28626					**Exon 3**	
251	**CRX probe** 11547-L16751				**Exon 3**		
256	**CRB1 probe** 06964-L06544			**Exon 7**			
265	**CRB1 probe** 20559-L20682			**Exon 6**			
272	**RPE65 probe** 20556-L14749						**Exon 12**
276	**RPE65 probe** 03602-L02969						**Exon 7**
283	**CRB1 probe** 06958-L06538			**Exon 2**			
290	**CRB1 probe** 06966-L06546			**Exon 9**			
297	Reference probe 05716-L05155	4p					
301	**RPE65 probe** 11548-L14340						**Exon 2**
310	**RPE65 probe** 03603-L02970						**Exon 9**
319	**LCA5 probe** 10271-L12159					**Exon 4**	
334	**RPE65 probe** 06952-L08634						**Exon 6**
341	**CRB1 probe** 06959-L16065			**Exon 3**			
347 Ø	**RPE65 probe** 11549-L12297						**Intron 10**
355	**AIPL1 probe** 06969-L06549		**Exon 1**				
361	Reference probe 05762-L17127	12q					
369	**RPE65 probe** 20639-L02968						**Exon 5**
381	Reference probe 22005-L31139	11q					
389	**LCA5 probe** 10273-L28627					**Exon 7**	
397	**CRB1 probe** 06968-L28628			**Exon 10**			
402	**CRB1 probe** 06960-L16753			**Exon 4**			
409	**CRX probe** 20558-L28673				**Exon 1**		
418	Reference probe 07593-L07278	21q					
427	**CRB1 probe** 06961-L06541			**Exon 5**			
434	**CRX probe** 20557-L14824				**Exon 4**		
439	**CRB1 probe** 06963-L20908			**Exon 6**			
445	**LCA5 probe** 20561-L21915					**Exon 8**	
454	**AIPL1 probe** 06970-L06550		**Exon 2**				
463	**LCA5 probe** 10274-L12166					**Exon 9**	
471	**LCA5 probe** 10272-L10783					**Exon 6**	
485	**CRB1 probe** 20560-L20684			**Exon 12**			
490	**LCA5 probe** 13714-L16617					**Exon 5**	
503	Reference probe 09870-L18172	2p					

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

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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. P221-C2 probes arranged according to chromosomal location

Table 2a. *RPE65*

Length (nt)	SALSA MLPA probe	*RPE65* exon[a]	Ligation site NM_000329.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*50-52 (Exon 1)*		
166	03599-L02966	Exon 1	46-45 reverse	ATAGACATTTTC-TTCCAGTTCAGG	1.2 kb
301	11548-L14340	Exon 2	69-70	CAGGGTTGAGCA-TCCTGCTGGTGG	1.9 kb
202	03600-L02967	Exon 3	232-233	TACCACCTGTTT-GATGGGCAAGCC	1.9 kb
238	20555-L14777	Exon 4	327-328	CGTACGGGCAAT-GACTGAGAAAAG	0.2 kb
369	20639-L02968	Exon 5	452-453	ATGCCCTTGTTA-ATGTCTACCCAG	3.8 kb
334	06952-L08634	Exon 6	687-688	CCCACCACTGCA-AGCAGGTGAGTT	1.2 kb
276	03602-L02969	Exon 7	726-727	GTCAGAGATCGT-TGTACAATTCCC	0.4 kb
178	06953-L06533	Exon 8	836-837	TTAACCTGTTCA-AGTTCCTTTCTT	0.3 kb
310	03603-L02970	Exon 9	992-993	TCTTCCATCACA-TCAACACCTATG	1.6 kb
	No probe	Exon 10			
347 Ø	11549-L12297	Intron 10	800 nt after exon 10	TATGAAGAATGA-GAAAGAGGGAGA	5.9 kb
196	06954-L06534	Exon 11	1266-1267	GCTGGAGCCTGA-AGTTCTCTTTTC	0.1 kb
272	20556-L14749	Exon 12	1326-1325 reverse	GTTTCCCACAAT-ACTTCTGGTAAT	0.2 kb
214	06956-L28625	Exon 13	1432-1433	TGGGTTTGGCAA-GAGCCTGATTCA	1.2 kb
148	04320-L28624	Exon 14	1553-1552 reverse	CAGAATCAGGAG-ATAAGCAGGCTT	
		Stop Codon	*1649-1651 (Exon 14)*		

Table 2b. *CRB1*

Length (nt)	SALSA MLPA probe	*CRB1* exon[a]	Ligation site NM_201253.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*162-164 (Exon 1)*		
208	06957-L20126	Exon 1	143-144	CACACCAGAGGA-TGTTCTCTAAAT	60.3 kb
283	06958-L06538	Exon 2	493-494	TGAAACTACCAT-TGGTTCCTGTGG	15.7 kb
341	06959-L16065	Exon 3	950-951	GAACTCAACACT-GATGAGTGTGCC	3.0 kb
402	06960-L16753	Exon 4	1074-1075	TGATGCCTCTTT-GTTGGTCAAAAC	9.5 kb
427	06961-L06541	Exon 5	1228-1229	GGAATGTGTGGA-GCTGTCCTCAGA	64.2 kb
265	20559-L20682	Exon 6	1436-1437	CATTGCCCATTT-GATAACCTTTCT	0.7 kb
439	06963-L20908	Exon 6	2093-2094	GGCTGTCTCCAA-GACATTAAAATT	5.8 kb
256	06964-L06544	Exon 7	2413-2414	ACCATCAGGCTT-ACTTCTAGCTTT	1.9 kb
190	07366-L06545	Exon 8	2882-2883	TCCCGGTGGGAT-GACTTCTCCTGT	5.3 kb
290	06966-L06546	Exon 9	3102-3103	CATTTGGTTTCA-GAACAAGGGATG	0.7 kb
142	06967-L06547	Exon 9	3796-3797	TGTGAACTGTGA-AGTGGATATAGA	3.1 kb
397	06968-L28628	Exon 10	3949-3950	TGGGAATGAGAA-GACAAATCTCAC	38.7 kb
	No probe	Exon 11			
485	20560-L20684	Exon 12	373 nt before exon 12	GAACTTTTGGCA-AGGGAGGAAAGA	
		Stop Codon	*4380-4382 (Exon 12)*		

Table 2c. *LCA5*

Length (nt)	SALSA MLPA probe	*LCA5* exon[a]	Ligation site NM_181714.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	613-615 (Exon 3)		
131	10266-L20190	Exon 1	198-197 reverse	CTCTTAGGACCA-AACTCCCAGCTG	11.9 kb
226 #	10267-L10779	Exon 2	95 nt before exon 2	AGGTGTAAAAGA-TGGGTCCTAGGA	6.3 kb
245	20562-L28626	Exon 3	460-461	AATGTGAATTGT-GGTCTACAAATA	5.5 kb
319	10271-L12159	Exon 4	975-974 reverse	TTGACCTGGAGT-TCAGATACTTCA	19.9 kb
490	13714-L16617	Exon 5	1363-1364	AACTGAGTACTA-ACAGTTTCCAAC	1.1 kb
471	10272-L10783	Exon 6	9 nt before exon 6 reverse	TTCCTGAAAACA-AACGCAATACAA	1.0 kb
389	10273-L28627	Exon 7	1599-1600	GCAGACCTGTGT-ACAAAAGGAGTA	2.5 kb
445	20561-L21915	Exon 8	1746-1745 reverse	AGAATCCCTGCT-TCTCCATGCCTG	2.0 kb
463	10274-L12166	Exon 9	2544-2543 reverse	TCTCTGCTGCCT-TTATTCCCAGGG	
		Stop Codon	2704-2706 (Exon 9)		

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.

Table 2d. *AIPL1*

Length (nt)	SALSA MLPA probe	*AIPL1* exon[a]	Ligation site NM_014336.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	18-20 (Exon 1)		
355	06969-L06549	Exon 1	23 nt before exon 1	GGAGGTGAGATT-ATCTCCGCCTGT	1.2 kb
454	06970-L06550	Exon 2	219-220	GAAACATGTTCA-AGCTCGAGGTCT	5.6 kb
220	06971-L06551	Exon 3	430-431	CGAGGACCTGGA-CGAGCTGCAGAA	1.4 kb
137	06972-L14818	Exon 4	585-586	ATCGGCTCTTCA-AGCTGGGCCGCT	0.2 kb
172	06973-L06553	Exon 5	696-697	TGAAGCTGGAGA-AGATGATCAATA	0.9 kb
153	06974-L06554	Exon 6	847-848	TCACGCAGAGGT-GTGGAATGAGGC	
		Stop Codon	1170-1172 (Exon 6)		

Table 2e. *CRX*

Length (nt)	SALSA MLPA probe	*CRX* exon[a]	Ligation site NM_000554.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	110-112 (Exon 2)		
409	20558-L28673	Exon 1	3-4	GATGTGTTTCCT-TCAGCCTCTGCT	12.6 kb
160	08771-L09336	Exon 2	185-186	GTGTGGATCTGA-TGCACCAGGCTG	2.0 kb
251	11547-L16751	Exon 3	160 nt after exon 3	CTGACCGCCTCA-TGGCTCTCATTG	4.5 kb
434	20557-L14824	Exon 4	2094-2095	GGTTAGGGACCT-TTCTAGAAATTC	
		Stop Codon	1007-1009 (Exon 4)		

[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.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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

P222 LCA mix-2 Leber congenital amaurosis, contains probes for the *GUCY2D*, *RDH12*, *RPGRIP1* and *CEP290* genes.

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 P221 LCA mix-1

- Bravo-Gil N et al. (2016). Improving the management of Inherited Retinal Dystrophies by targeted sequencing of a population-specific gene panel. *Sci Rep.* 6:23910.
- Corton M et al. (2013). High frequency of CRB1 mutations as cause of Early-Onset Retinal Dystrophies in the Spanish population. *Orphanet J Rare Dis.* 8:20.
- Kohn L et al. (2009). Breakpoint characterization of a novel approximately 59 kb genomic deletion on 19q13.42 in autosomal-dominant retinitis pigmentosa with incomplete penetrance. *Eur J Hum Genet.* 17:651-655.

P221 Product history	
Version	*Modification*
C2	Two reference probes have been replaced.
C1	One target probe has been removed and one reference probe has been replaced.
B1	The 88 and 96 nt DNA Denaturation control fragments have been replaced (QDX2). Probes for *LCA5* are now included. Additional target and reference probes have been added or replaced. Probes for *CFHR2* and *CFHR5* have been removed.
A1	First release.

Implemented changes in the product description
Version C2-02 –09 December 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *CRB1*, *AIPL1* and *LCA5* genes updated according to new versions of the NM_reference sequences. *Version C2-01 – 27 March 2019 (01P)* - Product description restructured, adapted to a new template and to a new product version (version number changed). - Ligation sites of the probes targeting the *RPE65* and *CRX* genes updated according to new versions of the NM_reference sequences. - Small changes of probe lengths in Table 1 and 2d in order to better reflect the true lengths of the amplification products. - Warning added for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene to Table 2c. - Several references were added.

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