Product Description
SALSA® MLPA® Probemix P497-A1 Opsin

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 10.

This SALSA MLPA probemix is for basic research and intended for experienced MLPA users only! This probemix enables you to quantify genes in the Opsin region on chromosome X. Since it will not provide you with clear cut answers in relationship with phenotype, interpretation of results can be complicated and we recommend to use results obtained by this probemix in combination with sequencing results. MRC Holland recommends thoroughly screening any available literature. Suggestions from specialists for improvement of this product or product description are highly appreciated.

Catalogue numbers:
- **P497-025R:** SALSA MLPA Probemix P497 Opsin, 25 reactions.
- **P497-050R:** SALSA MLPA Probemix P497 Opsin, 50 reactions.
- **P497-100R:** SALSA MLPA Probemix P497 Opsin, 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 P497 Opsin is a **research use only (RUO)** assay for the detection of deletions or duplications in the *OPN1LW* and *OPN1MW* genes and their locus control region (LCR). This probemix can also be used to detect the wild type sequence of the p.Cys203Arg (c.607T>C) mutation in *OPN1LW* and *OPN1MW*.

Copy number changes in the X-chromosomal genes *OPN1LW* and *OPN1MW* result in a number of cone photoreceptor disorders, including red-green colour blindness, deuteranopia, protanopia, X-linked cone dystrophy, blue cone monochromatism, and Bornholm eye disease. Red-green colour blindness is the most common form and affects males much more often than females. Among populations with Northern European ancestry, red-green colour blindness occurs in about 1 in 12 males and 1 in 200 females. Affected individuals have trouble distinguishing between shades of red, yellow, and green. The majority of affected individuals are thought to have a stable condition, however, some develop evidence of progression with increasing visual acuity loss, macular atrophy, retinal pigmentation, and electrophysiological evidence of S cone and rod involvement (Gardner et al. 2014; Gardner 2016).

The close proximity (~23 kb) and extensive sequence identity (96-98%) of the *OPN1LW* and *OPN1MW* genes promotes meiotic mispairing of the genes and non-homologous recombination, and less commonly gene conversion events. This might lead to the formation of a hybrid pigment gene that contains part of both *OPN1LW* and *OPN1MW*. Also, some unusual combinations of individually benign nucleotide variations in exon 3 that can arise from recombination between *OPN1LW* and *OPN1MW* are associated with vision disorders. These pathogenic SNP interchange haplotypes include variants abbreviated as LIAVA, LVAVA and MIAVA for the amino acids specified by positions p.153, p.171, p.174, p.178, and p.180 where L is leucine, V is valine, I is

isoleucine, A is alanine and M is methionine. These variants have shown to result in aberrant splicing and exon 3 skipping (Gardner et al. 2014; Greenwald et al. 2017; Verelli et al. 2004). Because of the frequent exchange, X chromosomes can have up to five *OPN1MW* copies, whereas individuals rarely have more than one *OPN1LW* copy per X chromosome.

Figure 1. Schematic representation of the position of the *OPN1LW* and *OPN1MW* genes on chromosome Xq28. Adapted from Gardner JC et al. (2009) via https://www.blueconemonochromacy.org/molecular-genetics/.

Transcription of the *OPN1LW* and *OPN1MW* genes is regulated by a nearby region of DNA, known as the LCR (Figure 1). Only the two opsin pigment genes nearest the LCR, generally the *OPN1LW* gene and the first copy of the *OPN1MW* gene, are active in the retina and contribute to colour vision. Figure 2 gives an overview of the copy number changes and variants in *OPN1LW* and *OPN1MW* related to disease.

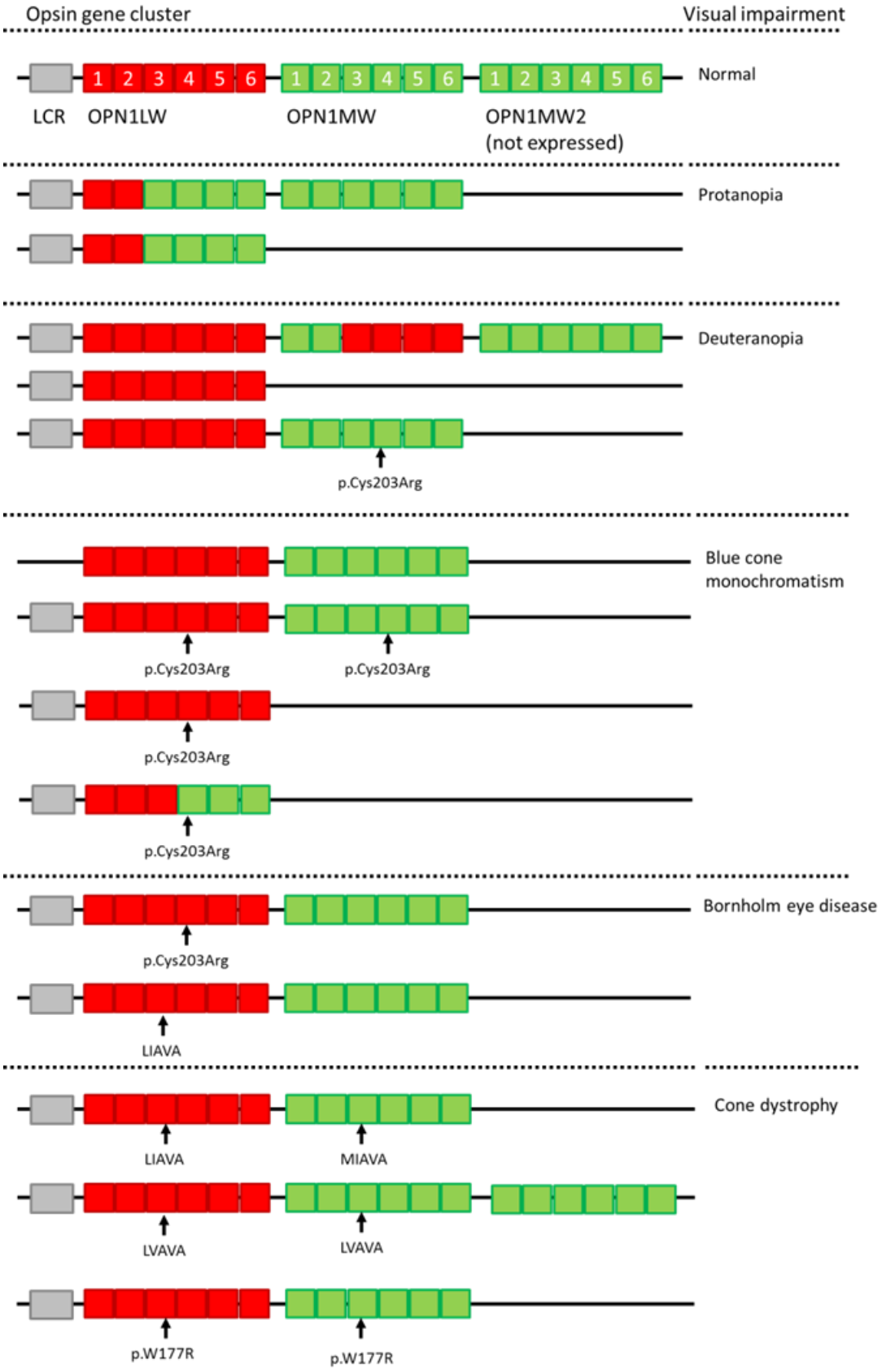

Figure 2. Changes in *OPN1LW* and *OPN1MW* leading to different visual disorders. Only the first amino acid of the exon 3 SNP interchangeable haplotypes (e.g. LIAVA) can be detected by this probemix, and the p.W177R mutation cannot be detected. See Interpretation of results for more information. Adapted from Haer-Wigman L. (personal communication).

The *OPN1LW* gene (6 exons) spans ~14.8 kb of genomic DNA and the *OPN1MW* gene (6 exons) spans ~14.3 kb of genomic DNA, both are located on chromosome Xq28.

More information is available at https://ghr.nlm.nih.gov/condition/color-vision-deficiency.

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 *OPN1LW* and *OPN1MW* exon numbering used in this P497-A1 Opsin product description is the exon numbering from the MANE project (release version 1.0) based on MANE Select transcripts NM_020061.6 and NM_000513.2, respectively, as indicated in Table 2. 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 MANE sequence. 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 P497-A1 Opsin contains 32 MLPA probes with amplification products between 130 and 364 nucleotides (nt). This includes six probes specific for the *OPN1LW* gene, six probes specific for the *OPN1MW* gene, two probes for the LCR and ten probes that detect both genes, including one probe that detects the wild type sequence of the p.Cys203Arg (c.607T>C) mutation (see Figure 3 in Interpretation of results). In addition, eight reference probes are included that detect locations on the X-chromosome. 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 male 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 reference 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 colour blindness. It is recommended to use samples of the same sex to facilitate interpretation and to use male reference samples with one copy of *OPN1LW* and one copy of *OPN1MW*. 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. The quality of cell lines can change; therefore samples should be validated before use.

SALSA Reference Selection DNA SD098
The selection of suitable reference DNA samples that can be used with P497 Opsin is complicated. To facilitate the selection of suitable reference DNA samples from your own sample collection, a Reference Selection DNA sample (catalogue number SD098) is provided with this probemix from MRC Holland. Reference Selection DNA SD098 is a male DNA sample with one copy of *OPN1LW* and one copy of *OPN1MW*. We recommend the use of SD098 for initial experiments on DNA samples from healthy individuals with the intention to select suitable reference samples. For further details, consult the Reference Selection DNA SD098 product description provided. **This product is for research use only (RUO).**

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
Figure 3 shows the location of the probes included in this probemix.

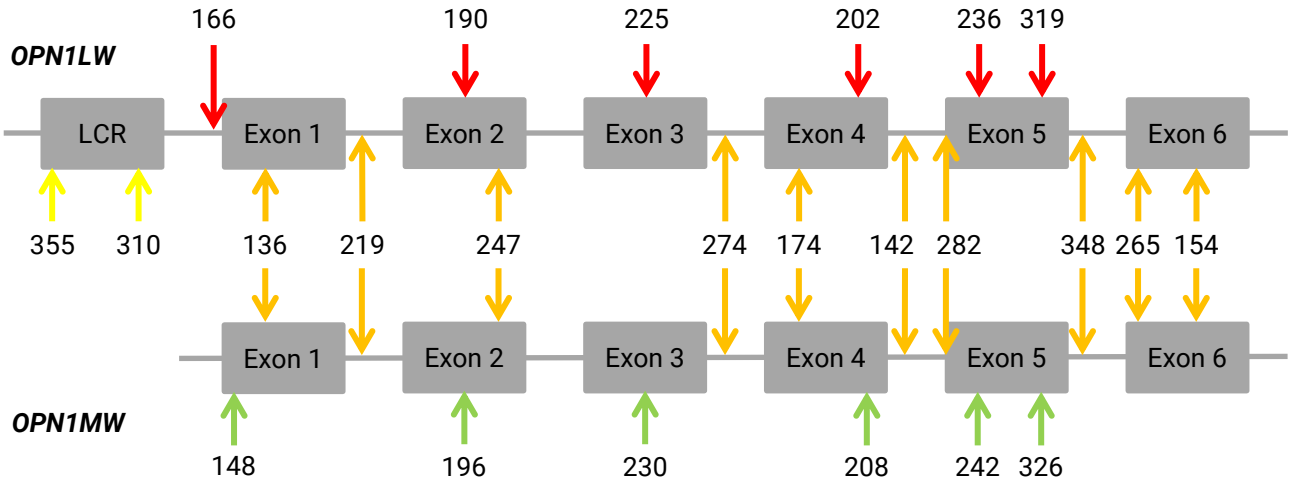

Figure 3. The location of probes targeting the genes *OPN1LW* (red) and *OPN1MW* (green) and the probes recognizing both genes (orange) and the LCR (yellow). The numbers above/below the arrows represent the length (in nt) of the respective probes.

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 table can be used to interpret MLPA results **when SALSA Reference Selection DNA SD098 or male reference samples with one copy of *OPN1LW* and one copy of *OPN1MW* are used**.

Theoretical expected ratios following normalization against Reference Selection DNA SD098 for different combinations of *OPN1LW* and *OPN1MW* copy numbers								
Sex	**Copy number**					**Ratio**		
	OPN1LW		*OPN1MW*		*OPN1LW/MW*	*OPN1LW*	*OPN1MW*	*OPN1LW/MW*
Male	1	+	1	=	2	1	1	1
	1	+	2	=	3	1	2	1.5
	1	+	3	=	4	1	3	2
	1	+	4	=	5	1	4	2.5
Female	2	+	2	=	4	1	1	1
	2	+	3	=	5	1	1.5	1.25
	2	+	4	=	6	1	2	1.5
	2	+	5	=	7	1	2.5	1.75
	2	+	6	=	8	1	3	2
	2	+	7	=	9	1	3.5	2.25
	2	+	8	=	10	1	4	2.5

It can be difficult to distinguish between higher *OPN1MW* copy numbers. Considering the different ratios for *OPN1LW*, *OPN1MW* and *OPN1LW/MW* together facilitates copy number determination.

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.)

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as gene conversions between *OPN1LW* and *OPN1MW* or changes observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results. See Table 2 for the probe order by chromosomal location for *OPN1LW* and *OPN1MW*.
- 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.

P497 specific notes:

- Deletion of a probe's recognition sequence on the X chromosome will lead to a complete absence of the corresponding probe amplification product in males, whereas female heterozygotes are recognizable by a 35-50% reduction in relative peak height. However, since X chromosomes have one to five *OPN1MW* copies and one copy of *OPN1LW*, this can differ per sample (see table on page 6). In addition, multiple probes detect both *OPN1LW* and *OPN1MW*.
- It is recommended to use reference samples with one copy of *OPN1LW* and one copy of *OPN1MW* to facilitate interpretation.
- Alterations in only one of the amino acids of the exon 3 SNP interchange haplotype are common in the normal population. Also, the probemix does not detect the full haplotype, for which sequence analysis is recommended.

Limitations of the procedure

- 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.

***OPN1LW* and *OPN1MW* mutation databases**

https://databases.lovd.nl/shared/genes/OPN1MW and https://databases.lovd.nl/shared/genes/OPN1LW. 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 to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P497-A1 Opsin

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*OPN1LW*	*OPN1MW*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 13499-L02104	Xp11		
136 »	**OPN1LW/MW probe** 23216-L32792		**Exon 1**	**Exon 1**
142 » Ø	**OPN1LW/MW probe** 23237-L32885		Intron 4	Intron 4
148	**OPN1MW probe** 21603-L30185			**Exon 1**
154 »	**OPN1LW/MW probe** 21608-L30190		**Exon 6**	**Exon 6**
160	Reference probe 21139-L29421	Xp22		
166	**OPN1LW probe** 21605-L31767		**Exon 1**	
174 » #	**OPN1LW/MW probe** 21606-L31768		**Exon 4**	**Exon 4**
184	Reference probe 13928-L15467	Xq22		
190	**OPN1LW probe** 21609-L32700		**Exon 2**	
196	**OPN1MW probe** 21609-L32702			**Exon 2**
202	**OPN1LW probe** 21611-L30198		**Exon 4**	
208	**OPN1MW probe** 21611-L30196			**Exon 4**
213	Reference probe 13203-L19630	Xq13		
219 » Ø	**OPN1LW/MW probe** 23217-L32886		Intron 1	Intron 1
225 ∞	**OPN1LW probe** 21613-L30201		**Exon 3**	
230 ∞	**OPN1MW probe** 21613-L30199			**Exon 3**
236	**OPN1LW probe** 21615-L30200		**Exon 5**	
242	**OPN1MW probe** 21615-L30202			**Exon 5**
247 »	**OPN1LW/MW probe** 23218-L32795		**Exon 2**	**Exon 2**
256	Reference probe 04994-L04380	Xq26		
265 »	**OPN1LW/MW probe** 23223-L32794		**Exon 6**	**Exon 6**
274 » Ø	**OPN1LW/MW probe** 23219-L32754		Intron 3	Intron 3
282 »	**OPN1LW/MW probe** 23221-L32759		**Exon 5**	**Exon 5**
301	Reference probe 07096-L08395	Xp22		
310 ^	**LCR probe** 21601-L30183		**LCR**	
319	**OPN1LW probe** 21602-L32704		**Exon 5**	
326	**OPN1MW probe** 21602-L32706			**Exon 5**
337	Reference probe 19394-L25801	Xp11		
348 » Ø	**OPN1LW/MW probe** 23222-L32753		Intron 5	Intron 5
355 ^	**LCR probe** 21612-L30197		**LCR**	
364	Reference probe 15361-L18375	Xq22		

[a] See section Exon numbering on page 4 for more information.

∞ Wild type sequence detected; c.457C (225 nt probe) = p.153L and c.457A (230 nt probe) = p.153M. Alterations in only one of the amino acids of the exon 3 SNP interchange haplotype are common in the normal population. Also, the probemix does not detect the full haplotype, for which sequence analysis is recommended.

» This probe detects a sequence that is present in both *OPN1LW* and *OPN1MW*.

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.

This probe detects the wild type sequence of the p.Cys203Arg (c.607T>C) mutation.

^ This probe is located in the locus control region (LCR).

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. P497-A1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
355 ^	21612-L30197	LCR	3.9 kb before exon 1 of *OPN1LW*	GTAGTCGCATTA-GAGACAAGTCCA	0.4 kb
310 ^	21601-L30183	LCR	3.5 kb before exon 1 of *OPN1LW reverse*	GCTCTGCTAATT-GATGATTAGGGC	3.5 kb
		OPN1LW	**NM_020061.6**		
		start codon	*42-44 (Exon 1)*		
166	21605-L31767	Exon 1	35 nt before exon 1	GCTGATCCCACA-GGCCAGTATAAA	0.1 kb
136 »	23216-L32792	Exon 1	50-51	GCCATGGCCCAG-CAGTGGAGCCTC	1.4 kb
219 » Ø	23217-L32886	Intron 1	1.3 kb after exon 1	TGCCCAGAATTT-CCTTGGTGGCAA	5.2 kb
190	21609-L32700	Exon 2	341-342	GTCGCTGACCTA-GCAGAGACCGTC	0.1 kb
247 »	23218-L32795	Exon 2	415-416	GGGCCACCCTAT-GTGTGTCCTGGA	2.1 kb
225 ∞	21613-L30201	Exon 3	498-499	GGGAGAGGTGGC-TGGTGGTCTGCA	0.5 kb
274 » Ø	23219-L32754	Intron 3	415 nt after exon 3	CAGCATTTCACT-GTTTTATCGACA	1.1 kb
174 » #	21606-L31768	Exon 4	648-647 *reverse*	GTCTGGGCCGCA-TGAAGTCTTCAG	0.1 kb
202	21611-L30198	Exon 4	747-748	TCGCTATCATCA-TGCTCTGCTACC	0.4 kb
142 » Ø	23237-L32885	Intron 4	377 nt after exon 4	AGCAAATGACAC-CTGCAAATCAAG	1.2 kb
282 »	23221-L32759	Exon 5	1 nt before exon 5	CTGTCTCCCTTA-GGTGGCAAAGCA	0.1 kb
236	21615-L30200	Exon 5	871-872	GATCTTTGCGTA-CTGCTTCTGCTG	0.1 kb
319	21602-L32704	Exon 5	967-968	CCTGCCGGCCTA-CTTTGCCAAAAG	0.5 kb
348 » Ø	23222-L32753	Intron 5	399 nt after exon 5	TCTGTTCAGACT-CGACCCTTCTCA	1.9 kb
265 »	23223-L32794	Exon 6	1034-1035	CAGTTTCGAAAC-TGCATCTTGCAG	0.1 kb
154 »	21608-L30190	Exon 6	1124-1125	GTGTCCTCGGTA-TCGCCTGCATGA	
		stop codon	*1134-1136 (Exon 6)*		
		OPN1MW	**NM_000513.2**		
		start codon	*83-85 (Exon 1)*		
148	21603-L30185	Exon 1	21-22	CGGTATAAAGCA-CCGTGACCCTCA	0.1 kb
136 »	23216-L32792	Exon 1	91-92	GCCATGGCCCAG-CAGTGGAGCCTC	1.4 kb
219 » Ø	23217-L32886	Intron 1	1.3 kb after exon 1	TGCCCAGAATTT-CCTTGGTGGCAA	3.9 kb
196	21609-L32702	Exon 2	382-383	GTCGCTGACCTG-GCAGAGACCGTC	0.1 kb
247 »	23218-L32795	Exon 2	456-457	GGGCCACCCTAT-GTGTGTCCTGGA	2.1 kb
230 ∞	21613-L30199	Exon 3	539-540	GGGAGAGATGGA-TGGTGGTCTGCA	0.5 kb
274 » Ø	23219-L32754	Intron 3	415 nt after exon 3	CAGCATTTCACT-GTTTTATCGACA	1.1 kb
174 » #	21606-L31768	Exon 4	689-688 *reverse*	GTCTGGGCCGCA-TGAAGTCTTCAG	0.1 kb
208	21611-L30196	Exon 4	788-789	TCAGCATCATCG-TGCTCTGCTACC	0.4 kb
142 » Ø	23237-L32885	Intron 4	377 nt after exon 4	AGCAAATGACAC-CTGCAAATCAAG	1.2 kb
282 »	23221-L32759	Exon 5	1 nt before exon 5	CTGTCTCCCTTA-GGTGGCAAAGCA	0.1 kb
242	21615-L30202	Exon 5	912-913	GGTCCTGGCATT-CTGCTTCTGCTG	0.1 kb
326	21602-L32706	Exon 5	1008-1009	CCTGCCGGCCTT-CTTTGCCAAAAG	0.5 kb
348 » Ø	23222-L32753	Intron 5	399 nt after exon 5	TCTGTTCAGACT-CGACCCTTCTCA	1.9 kb
265 »	23223-L32794	Exon 6	1075-1076	CAGTTTCGAAAC-TGCATCTTGCAG	0.1 kb
154 »	21608-L30190	Exon 6	1165-1166	GTGTCCTCGGTA-TCGCCTGCATGA	
		stop codon	*1175-1177 (Exon 6)*		

[a] See section Exon numbering on page 4 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.

∞ Wild type sequence detected; c.457C (225 nt probe) = p.153L and c.457A (230 nt probe) = p.153M. Alterations in only one of the amino acids of the exon 3 SNP interchange haplotype are common in the normal population. Also, the probemix does not detect the full haplotype, for which sequence analysis is recommended.

» Shaded boxes. This probe detects a sequence that is present in both *OPN1LW* and *OPN1MW*.

This probe detects the wild type sequence of the p.Cys203Arg (c.607T>C) mutation.

^ This probe is located in the locus control region (LCR).

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.

References

- Gardner JC et al. (2009). Blue cone monochromacy: Causative mutations and associated phenotypes. *Mol Vision*. 15:876-884.
- Gardner JC et al. (2014). Three different cone opsin gene array mutational mechanisms with genotype–phenotype correlation and functional investigation of cone opsin variants. *Hum Mutat*. 35:1354-1362.
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- Greenwald SH et al. (2017). Role of a dual splicing and amino acid code in myopia, cone dysfunction and cone dystrophy associated with L/M opsin interchange mutations. *Transl Vis Sci Technol*. 6:2.
- 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.
- Verrelli BC et al. (2004). Signatures of selection and gene conversion associated with human color vision variation. *Americ J of Hum Genet*. 75:363-375.

Selected publications using SALSA MLPA Probemix P497 Opsin

The studies below used the test version of SALSA MLPA Probemix P497 Opsin (SALSA MLPA Probemix X080 Opsin).

- Haer-Wigman L et al. (2022). Diagnostic analysis of the highly complex *OPN1LW*/*OPN1MW* gene cluster using long-read sequencing and MLPA. *NPJ Genom Med*. 7:65.
- Stingl K et al. (2022). Novel *OPN1LW*/*OPN1MW* exon 3 haplotype-associated splicing defect in patients with X-linked cone dysfunction. *Int J Mol Sci*. 23:6868.
- Wissinger B et al. (2022). The landscape of submicroscopic structural variants at the *OPN1LW*/*OPN1MW* gene cluster on Xq28 underlying blue cone monochromacy. *Proc Natl Acad Sci U S A*. 119:e2115538119.

P497 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-01 – 11 December 2023 (04P) - Not applicable, new document.

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