Product Description
SALSA® MLPA® Probemix P151-C1/P152-C2 ABCA4

To be used with the MLPA General Protocol.

P151 version C1
For complete product history see page 9.
P152 version C2
As compared to version C1, two reference probes have been removed, three reference probes have been replaced and six probe lengths have been adjusted. For complete product history see page 9.

Catalogue numbers:
- **P151-025R:** SALSA MLPA Probemix ABCA4 mix-1, 25 reactions.
- **P151-050R:** SALSA MLPA Probemix ABCA4 mix-1, 50 reactions.
- **P151-100R:** SALSA MLPA Probemix ABCA4 mix-1, 100 reactions.
- **P152-025R:** SALSA MLPA Probemix ABCA4 mix-2, 25 reactions.
- **P152-050R:** SALSA MLPA Probemix ABCA4 mix-2, 50 reactions.
- **P152-100R:** SALSA MLPA Probemix ABCA4 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 Probemixes P151 ABCA4 mix-1 and P152 ABCA4 mix-2 are **research use only (RUO)** assays for the detection of deletions or duplications in the *ABCA4* gene, which is associated with several diseases, including Stargardt macular dystrophy, retinitis pigmentosa type 19 (RP19), cone-rod dystrophy type 3, early-onset severe retinal dystrophy, fundus flavimaculatus, and age-related macular degeneration type 2 (ARMD2). These diseases are inherited in an autosomal recessive manner, except from ARMD2 which is a dominant disorder. The *ABCA4* gene encodes an ATP-binding cassette (ABC) transporter. This transmembrane protein, expressed exclusively in retinal photoreceptors, is involved in energy-dependent transport of a wide spectrum of substrates across membranes.

More information is available at http://omim.org/entry/601691.

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 *ABCA4* exon numbering used in this P151-C1/P152-C2 ABCA4 product description is the exon numbering from the NG_009073.1 sequence. 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 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 P151-C1 ABCA4 mix-1 contains 39 MLPA probes with amplification products between 130 and 454 nucleotides (nt). This includes 26 probes for the *ABCA4* gene and one downstream flanking probe in the *BCAR3* gene. In addition, 12 reference probes are included that detect autosomal chromosomal locations. The SALSA MLPA Probemix P152-C2 ABCA4 mix-2 contains 33 MLPA probes with amplification products between 133 and 445 nucleotides (nt). This includes 23 probes for the *ABCA4* gene. In addition, ten reference probes are included that detect autosomal chromosomal locations. Together, these probemixes cover all exons of the *ABCA4* gene with the exception of exon 13, 33 and 40. 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 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 hereditary eye or vision abnormalities. 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.

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 *ABCA4* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemixes P151/P152 ABCA4.
- 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.

***ABCA4* mutation database**

https://databases.lovd.nl/shared/genes/ABCA4. 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 *ABCA4* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1a. SALSA MLPA Probemix P151-C1 ABCA4 mix-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*ABCA4*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L19287	5q	
136	Reference probe 21270-L28956	1p	
140 Ø	**ABCA4 probe** 17132-L20324		**Intron 32**
148	**ABCA4 probe** 17135-L20327		**Exon 25**
154	**ABCA4 probe** 17136-L20328		**Exon 42**
160	Reference probe 21308-L29714	3p	
166	**ABCA4 probe** 04932-L04318		**Exon 3**
172	**ABCA4 probe** 04948-L04334		**Exon 19**
178	**ABCA4 probe** 04963-L04349		**Exon 35**
190	Reference probe 12422-L13423	14q	
196	**ABCA4 probe** 04934-L04747		**Exon 5**
203	**ABCA4 probe** 04950-L04977		**Exon 21**
211	**ABCA4 probe** 04965-L04351		**Exon 37**
220	Reference probe 21057-L30157	10q	
229	**ABCA4 probe** 04936-L04322		**Exon 7**
238	**ABCA4 probe** 04952-L04338		**Exon 23**
247	**ABCA4 probe** 04967-L04353		**Exon 39**
256	Reference probe 07372-L07019	8q	
266	**ABCA4 probe** 05585-L20688		**Exon 32**
274	**ABCA4 probe** 17140-L20332		**Exon 15**
283	**ABCA4 probe** 17143-L20335		**Exon 27**
293	**ABCA4 probe** 04968-L04354		**Exon 41**
300	Reference probe 16027-L18204	12p	
310	**ABCA4 probe** 04940-L04326		**Exon 11**
328	**ABCA4 probe** 04969-L04748		**Exon 43**
337	Reference probe 13869-L15387	7p	
346	**ABCA4 probe** 17150-L20342		**Exon 1**
355	**ABCA4 probe** 17151-L20343		**Exon 17**
364	**ABCA4 probe** 04971-L04749		**Exon 45**
373	Reference probe 00546-L01247	11q	
382	Reference probe 16885-L19718	16q	
391	**ABCA4 probe** 17152-L20344		**Exon 9**
400	**ABCA4 probe** 04960-L04346		**Exon 31**
409	**ABCA4 probe** 21009-L29227		**Exon 2**
418 ¬	BCAR3 probe 00714-L00140		Downstream
427	**ABCA4 probe** 17156-L20348		**Exon 29**
436	**ABCA4 probe** 17157-L20349		**Exon 34**
445	Reference probe 10709-L11291	6p	
454	Reference probe 13348-L14774	18q	

[a] See section Exon numbering on page 1 for more information.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
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.

Table 1b. SALSA MLPA Probemix P152-C2 ABCA4 mix-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*ABCA4*
64-105	Control fragments – see table in probemix content section for more information		
133	Reference probe 16316-L20697	3q	
142	**ABCA4 probe** 17133-L20325		**Exon 28**
148	Reference probe 14279-L15949	15q	
154	**ABCA4 probe** 04947-L04333		**Exon 18**
160	**ABCA4 probe** 17137-L20329		**Exon 20**
166	**ABCA4 probe** 04933-L04319		**Exon 4**
178 ¥	**ABCA4 probe** 04970-L32790		**Exon 44**
190	Reference probe 12422-L13423	14q	
209	**ABCA4 probe** 04972-L04358		**Exon 46**
217	Reference probe 14388-L16375	5q	
224	**ABCA4 probe** 17139-L20939		**Exon 47**
230	**ABCA4 probe** 04937-L20948		**Exon 8**
238	**ABCA4 probe** 04953-L04339		**Exon 24**
246	**ABCA4 probe** 04964-L04350		**Exon 36**
255	Reference probe 09899-L10312	16p	
274	**ABCA4 probe** 04939-L04325		**Exon 10**
283	**ABCA4 probe** 17141-L20333		**Exon 50**
292	**ABCA4 probe** 17144-L20336		**Exon 38**
301	Reference probe 14941-L16674	6q	
310	**ABCA4 probe** 17145-L20337		**Exon 6**
328	**ABCA4 probe** 17147-L20339		**Exon 22**
337 *	Reference probe 19093-L24980	4q	
345 ¥	**ABCA4 probe** 17148-L32798		**Exon 26**
355 ¥	**ABCA4 probe** 04943-L32799		**Exon 14**
363 ¥	**ABCA4 probe** 04959-L32800		**Exon 30**
373 ¥	**ABCA4 probe** 04973-L32801		**Exon 48**
382 *	Reference probe 21221-L29596	9p	
400 ¥	**ABCA4 probe** 04945-L32802		**Exon 16**
409	**ABCA4 probe** 17153-L20345		**Exon 49**
417	Reference probe 13817-L15311	2q	
427	**ABCA4 probe** 17154-L20346		**Exon 12**
436 Ø	**ABCA4 probe** 17158-L20350		**Intron 1**
445 *	Reference probe 09612-L09907	20p	

[a] See section Exon numbering on page 1 for more information.

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.
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.

Table 2. *ABCA4* probes arranged according to chromosomal location

Length (nt) P151	Length (nt) P152	SALSA MLPA probe	*ABCA4* exon[a]	Ligation site NM_000350.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*104-106 (Exon 1)*		
346		17150-L20342	Exon 1	33-34	AGGCGCTCTTAA-CGGCGTTTATGT	0.8 kb
	436 Ø	17158-L20350	Intron 1	680 nt after exon 1	CTGGAGCCCTAA-AAGCACGACACA	7.3 kb
409		21009-L29227	Exon 2	185-186	GCTTTGTGGTGG-AACTCGTGTGGC	1.6 kb
166		04932-L04318	Exon 3	358-359	TTTCAAAGCCCC-ACCCCAGGAGAA	2.8 kb
	166	04933-L04319	Exon 4	477-478	CCTTGGCCGTAT-TTGGACAGAGCT	5.6 kb
196		04934-L04747	Exon 5	633-634	GTCTGACTCAGT-GGTCTACCTTCT	4.4 kb
	310	17145-L20337	Exon 6	110 nt after exon 6	GGACAATCCTTG-CAAAAGCTCCAG	15.3 kb
229		04936-L04322	Exon 7	895-896	CTAGACAGCCGT-TCTCAAGGTATC	2.9 kb
	230	04937-L20948	Exon 8	1126-1127	AACTGGTATGAA-GACAATAACTAT	1.0 kb
391		17152-L20344	Exon 9	68 nt before exon 9	TGATGTGGATAC-ACTAATGACTAT	0.9 kb
	274	04939-L04325	Exon 10	1399-1400	AAAGCCTGGGAA-GAAGTAGGGCCC	0.8 kb
310		04940-L04326	Exon 11	1550-1551	TAAACTTCCTCT-ACAAGGGCCCTC	14.4 kb
	427	17154-L20346	Exon 12	118 nt before exon 12	AGTTTATAGGCC-TCCTTGCATTTG	0.9 kb
		No probe	Exon 13			
	355	04943-L32799	Exon 14	2216-2217	TCCTGGACAGCT-TCTCCATCATGT	3.8 kb
274		17140-L20332	Exon 15	2294-2295	ACAGCGACCCAT-TCATCCTCTTCC	1.6 kb
	400	04945-L32802	Exon 16	2637-2638	GATGATGCTCCT-TGATGCTGCTGT	3.7 kb
355		17151-L20343	Exon 17	163 nt after exon 17	TTGCAGCGTCTG-GGGGACCACCTT	2.5 kb
	154	04947-L04333	Exon 18	2761-2762	GGTGCAGGGTGT-TCAACCAGAGAA	1.9 kb
172		04948-L04334	Exon 19	2871-2872	ACGTGAGCATCC-AGGGTGGGTTCC	2.4 kb
	160	17137-L20329	Exon 20	3118-3119	CGGCAGAGCCTT-GGCATGTGTCCA	1.3 kb
203		04950-L04977	Exon 21	3239-3240	TGGAAGCCATGT-TGGAGGACACAG	0.4 kb
	328	17147-L20339	Exon 22	90 nt before exon 22	CACTGCTGGGTT-AAGGTACCCCCA	1.7 kb
238		04952-L04338	Exon 23	3521-3522	GAAGGCTCTACT-GCTCAGGCACCC	1.2 kb
	238	04953-L04339	Exon 24	3665-3666	TCTCCACCACGT-GTCCAGCCCACG	2.8 kb
148		17135-L20327	Exon 25	3759-3760	TGTTCCAGAGGC-AAAGCTGGTGGA	0.6 kb
	345	17148-L32798	Exon 26	55 nt after exon 26	TCCCAAGTTCCA-TCTCGAAAGTCT	5.0 kb
283		17143-L20335	Exon 27	68 nt after exon 27	CTCCTCTCCTGA-GTGTTCCTTCAG	0.6 kb
	142	17133-L20325	Exon 28	1 nt before exon 28	TATTCTCCCACA-GATCGTGCTCCC	0.8 kb
427		17156-L20348	Exon 29	85 nt after exon 29	AGGCGTTGGGGA-GGCCCCACTCAA	0.8 kb
	363	04959-L32800	Exon 30	4521-4522	CATCACCCAGCT-GTTCCAGAAGCA	4.6 kb
400		04960-L04346	Exon 31	4692-4693	GGACAGGAACAT-CTCCGACTTCTT	1.6 kb
266		05585-L20688	Exon 32	11 nt before exon 32	CATTATTTTATT-TTGGCTTTCAGC	1.1 kb
140 Ø		17132-L20324	Intron 32	404 nt before exon 33	TGTGAAAGAGGA-CCTGGAGTTGGC	0.8 kb
		No probe	Exon 33			
436		17157-L20349	Exon 34	108 nt after exon 34	CTCCTGCTGAAA-TCTAGCAAGGAA	0.2 kb
178		04963-L04349	Exon 35	5073-5074	AATCACCGTCAT-TAGCCAACCCCT	1.7 kb
	246	04964-L04350	Exon 36	5256-5257	TATCAGTGGAGT-GAGCCCCACCAC	3.8 kb
211		04965-L04351	Exon 37	5339-5340	TGGTGGGCATCT-TCATCGGGTTTC	1.1 kb
	292	17144-L20336	Exon 38	25 nt before exon 38	GGAACTCCATCA-CTCTGAGTTGTC	3.4 kb
247		04967-L04353	Exon 39	5591-5592	ACGCCGTGCTGA-GGAAGCTGCTCA	0.2 kb
		No probe	Exon 40			
293		04968-L04354	Exon 41	5838-5839	GCCCACTAAGGA-GCCCATTGTTGA	0.7 kb
154		17136-L20328	Exon 42	35 nt after exon 42	TGAAAGGCTTCC-GAACATCAGCTC	0.5 kb
328		04969-L04748	Exon 43	6072-6073	TGGGGACACCAC-AGTGACCTCAGG	2.2 kb
	178	04970-L32790	Exon 44	6202-6203	CGAGAACATCTT-TACCTTTATGCC	3.5 kb
364		04971-L04749	Exon 45	6286-6287	CTGGGCCTGACT-GTCTACGCCGAC	0.9 kb
	209	04972-L04358	Exon 46	6478-6479	GCTGTGGTCCTC-ACATCCCACAGG	0.2 kb
	224	17139-L20939	Exon 47	10 nt after exon 47	AAGTAAGCAGAT-GGTGGGGCGTGC	2.8 kb
	373	04973-L32801	Exon 48	6717-6718	GCACTACAACAT-GCTCCAGTTCCA	1.6 kb
	409	17153-L20345	Exon 49	184 nt before exon 49	TCTAAGAAGCTA-GCTCTGACCAGG	3.1 kb

Length (nt) P151	Length (nt) P152	SALSA MLPA probe	*ABCA4* exon[a]	Ligation site NM_000350.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
	283	17141-L20333	Exon 50	6921-6922	CCTTCCACAGGA-CTGATCTTTCAC	410.8 kb
			stop codon	6925-6927 (Exon 50)		
418 ¬		00714-L00140	*BCAR3* gene		TGGAGACTGTCT-CCTCCTTCAGGC	

[a] See section Exon numbering on page 1 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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
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

- P235 Retinitis: Contains probes for the autosomal genes *IMPDH1*, *PRPF31*, *RHO* and *RP1*.
- P366 CHM-RP2-RPGR: Contains probes for the X-linked genes *CHM*, *RP2* and *RPGR*.

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 P151/P152 ABCA4

- Aquirre-Lamban J et al. (2009). Molecular analysis of the ABCA4 gene for reliable detection of allelic variations in Spanish patients: identification of 21 novel variants. *Br J Ophthalmol*. 93:641-621.
- Bax NM et al. (2015). Heterozygous deep-intronic variants and deletions in ABCA4 in persons with retinal dystrophies and one exonic ABCA4 variant. *Hum Mutat*. 36:43-47.
- Falfoul Y et al. (2018). Phenotypic progression of stargardt disease in a large consanguineous Tunisian family harboring new ABCA4 mutations. *J Ophthalmol*. 2018.
- Jiang F et al. (2016). Screening of ABCA4 gene in a Chinese cohort with Stargardt disease or cone-rod dystrophy with a report on 85 novel mutations. *Invest Ophthalmol Vis Sci*. 57: 153-161.
- Liu X et al. (2021). Molecular diagnosis based on comprehensive genetic testing in 800 Chinese families with non-syndromic inherited retinal dystrophies. *Clin Exp Ophthalmol*. 49:46-59.
- Riveiro-Alvarez R et al. (2007). Partial paternal uniparental disomy (UPD) of chromosome 1 in a patient with Stargardt disease. *Mol Vis*. 26:96-101.
- Riveiro-Alvarez R et al. (2013). Outcome of ABCA4 disease-associated alleles in autosomal recessive retinal dystrophies: retrospective analysis in 420 Spanish families. *Am J Ophthalmol*. 120:2332-2337.
- Rodríguez-Muñoz A et al. (2020). Expanding the clinical and molecular heterogeneity of nonsyndromic inherited retinal dystrophies. *J Mol Diagn*. 22:532-543.
- Smaragda K et al. (2018). Mutation spectrum of the ABCA4 gene in a Greek cohort with Stargardt disease: identification of novel mutations and evidence of three prevalent mutated alleles. *J Ophthalmol*. 2018.
- Wawrocka A et al. (2018). Novel variants identified with next-generation sequencing in Polish patients with cone-rod dystrophy. *Mol Vis*. 24:326.

P151 product history	
Version	*Modification*
C1	One ABCA4 probe has been removed, three reference probes have been replaced and one probe length has been adjusted.
B1	Eight ABCA4 probes and ten reference probes have been replaced, two new ABCA4 probes have been included and the QDX2 control fragments have been added.
A1	First release.

P152 product history	
Version	*Modification*
C2	Two reference probes have been removed, three reference probes have been replaced and six probe lengths have been adjusted.
C1	Two ABCA4 probes and two reference probes have been removed.
B2	A sequence problem in the 224 nt exon 47 probe has been solved.
B1	Ten ABCA4 probes and all reference probes have been replaced (SNPs), two new ABCA4 probes have been included and the QDX2 control fragments have been added.
A1	First release.

Implemented changes in the product description

Version P151-C1/P152-C2-01 – 04 November 2021 (04P)
- 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 *ABCA4* gene updated according to new version of the NM_ reference sequence.
- The notation in Table 1a and 2 has been changed from exon to intron for two probes (17132-L20324; 17158-L20350), based on their distance from the exon. This is only a change in notation; the sequence detected by the probes has not been changed.
- The probemix content section has also been updated to include that there is no probe for exon 33.
- Small changes of probe lengths in Table 1a, 1b and 2 in order to better reflect the true lengths of the amplification products.

Version C1-01 – 13 April 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to new product versions (version numbers changed, lot number added, changes in Table 1 and Table 2).

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