Product Description
SALSA® MLPA® Probemix P367-A3 BEST1-PRPH2

To be used with the MLPA General Protocol.

Version A3
For complete product history see page 7.

Catalogue numbers:
- **P367-025R:** SALSA MLPA Probemix P367 BEST1-PRPH2, 25 reactions.
- **P367-050R:** SALSA MLPA Probemix P367 BEST1-PRPH2, 50 reactions.
- **P367-100R:** SALSA MLPA Probemix P367 BEST1-PRPH2, 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 P367 BEST1-PRPH2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *BEST1* and *PRPH2* genes, which are associated with Vitelliform macular dystrophy.

Vitelliform macular dystrophy is a genetic eye disorder that can cause progressive vision loss. This disorder affects the retina, the specialized light-sensitive tissue that lines the back of the eye. The early-onset form (known as Best disease) usually appears in childhood; both the onset of symptoms and the severity of vision loss vary widely. The adult-onset form starts mid-adulthood, and tends to cause vision loss that worsens slowly over time. The two forms of vitelliform macular dystrophy each cause characteristic changes in the macula that can be detected during an eye examination. Defects in the gene *BEST1* (=*VMD2*) on chromosome 11 are responsible for Best disease and for some cases of the adult-onset form of vitelliform macular dystrophy. Changes in the *PRPH2* (=*RDS*) gene on chromosome 6 can also cause the adult-onset form of vitelliform macular dystrophy.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1167/

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 *BEST1 and PRPH2* exon numbering used in this P367-A3 BEST1-PRPH2 description is the exon numbering from the NG_009033.1 and NG_009176.2 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 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 P367 BEST1-PRPH2 contains 24 MLPA probes with amplification products between 130 and 274 nucleotides (nt). This includes eleven probes for the *BEST1* gene, one probe for each exon, and five probes for the *PRPH2* gene, with one probe for exon 1 and exon 3, two probes for exon 2 and one additional probe targeting intron 1. In addition, eight reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique

The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation

Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes 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 Vitelliform macular dystrophy. 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 *BEST1 and PRPH2* genes are small (point) mutations, none of which will not be detected by using SALSA MLPA Probemix P367-A3 BEST1-PRPH2.
- 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.

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 SNVs and unusual results (e.g., a duplication of *BEST1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P367-A3 BEST1-PRPH2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*BEST1*	*PRPH2*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L13645	5q		
136	**BEST1** probe 06940-L14703		**Exon 8**	
143 Ø	**PRPH2** probe 12625-L14441			**Intron 1**
148	Reference probe 00798-L00316	13q		
154	**BEST1** probe 06933-L31234		**Exon 1**	
160	Reference probe 20053-L27397	16q		
166	**BEST1** probe 13183-L06515		**Exon 3**	
172	**PRPH2** probe 06929-L07114			**Exon 2**
178	**BEST1** probe 13184-L06516		**Exon 4**	
184	Reference probe 08788-L08812	10q		
192	**BEST1** probe 06941-L06521		**Exon 9**	
196	**BEST1** probe 06937-L14704		**Exon 5**	
202	**PRPH2** probe 06928-L13648			**Exon 1**
208	**BEST1** probe 06934-L06514		**Exon 2**	
214	Reference probe 09103-L09162	4q		
221	**BEST1** probe 06943-L06523		**Exon 11**	
227	**PRPH2** probe 13185-L13649			**Exon 3**
232	Reference probe 16429-L18882	18q		
237	**BEST1** probe 13186-L13711		**Exon 6**	
244	**PRPH2** probe 13187-L13791			**Exon 2**
250	**BEST1** probe 13188-L06519		**Exon 7**	
256	Reference probe 02469-L01913	15q		
265	**BEST1** probe 13189-L06522		**Exon 10**	
274	Reference probe 12782-L30989	2q		

[a] See section Exon numbering on page 1 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. P367-A3 probes arranged according to chromosomal location

Table 2a. *BEST1*

Length (nt)	SALSA MLPA probe	*BEST1* exon[a]	Ligation site NM_004183.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*114-116 (Exon 2)*		
154	06933-L31234	Exon 1	53-52 reverse	GGTGGGTCCGAT-GATCCCACAGAA	1.5 kb
208	06934-L06514	Exon 2	243-244	TGCTCTGCTACT-ACATCATCCGCT	3.2 kb
166	13183-L06515	Exon 3	297-298	AACAGCTGATGT-TTGAGAAACTGA	0.6 kb
178	13184-L06516	Exon 4	382-383	GACGCTGGTCGT-GACCCGCTGGTG	1.2 kb
196	06937-L14704	Exon 5	652-653	ACCACACAACAT-GTTCTGGGTGCC	0.7 kb
237	13186-L13711	Exon 6	146 nt after exon 6	TCTGGACTTTGA-AGTGCCAAGTTC	0.7 kb
250	13188-L06519	Exon 7	967-968	CTTCTTCTATGT-TGGCTGGCTGAA	1.2 kb
136	06940-L14703	Exon 8	1028-1027 reverse	CAGTTGGTCTCA-AAATCATCATCA	0.4 kb
192	06941-L06521	Exon 9	1099-1100	GCACCAGGACCT-GCCTCGGATGGA	2.4 kb
265	13189-L06522	Exon 10	1265-1266	GACGAGGAGGAT-GCTCACGCTGGC	2.1 kb
221	06943-L06523	Exon 11	2145-2144 reverse	GCTGTCTTGGAT-TCAGATAGAACT	
		Stop Codon	*1869-1871 (Exon 11)*		

Table 2b. *PRPH2*

Length (nt)	SALSA MLPA probe	*PRPH2* exon[a]	Ligation site NM_000322.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*264-266 (Exon 1)*		
202	06928-L13648	Exon 1	774-775	GCAACAACGGTT-TTCGGGACTGGT	3.3 kb
143 Ø	12625-L14441	Intron 1	3186 nt after exon 1	CTGCAGTGGGCT-ACGTGTTCTTCC	14.0 kb
172	06929-L07114	Exon 2	873-874	TGGATGGGCGGT-ACCTGGTGGACG	0.2 kb
244	13187-L13791	Exon 2	1066-1067	CATGGGTGTCGT-CACGCTCCTCAT	6.3 kb
227	13185-L13649	Exon 3	1540-1541	GTGAAGCTCCCT-TCAGGCCCGCTG	
		Stop Codon	*1302-1304 (Exon 3)*		

[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.

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

- P219 PAX6 Contains probes for the *PAX6*, *SOX2*, *WT1* genes, involved in multiple ocular malformations.
- P221 LCA1 Contains probes for the *AIPL1*, *CRB1*, *CRX*, *RPE65* genes, associated with Leber Congenital Amaurosis.
- P222 LCA2 Contains probes for the *GUCY2D*, *RDH12*, *RPGRIP1*, *CEP290* genes, associated with Leber Congenital Amaurosis.
- P229 OPA1 Contains probes for the *OPA1* gene, associated with optic atrophy type 1.

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 P367 BEST1-PRPH2

- Tian L et al. (2017). Screening of *BEST1* Gene in a Chinese Cohort With Best Vitelliform Macular Dystrophy or Autosomal Recessive Bestrophinopathy. *Invest Ophthalmol Vis Sci.* 58(9):3366-3375.
- Boulanger-Scemama E et al. (2015). Next-generation sequencing applied to a large French cone and cone-rod dystrophy cohort: mutation spectrum and new genotype-phenotype correlation. *Orphanet J Rare Dis.* 10:85.

P367 product history	
Version	*Modification*
A3	Two reference probes have been replaced and one probe length has been adjusted.
A2	Compared to previous lot, two reference probes have been replaced and the control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description
Version A3-02 – 23 February 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *BEST1* and *PRPH2* genes updated according to new version of the NM_ reference sequences. *Version A3-01 – 28 June 2018 (01P)* - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). *Version 08 – 02 June 2017 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - New reference added. - Manufacturer’s address adjusted. - “Peak area” replaced with “peak height”. - Various minor textual and layout changes.

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