Product Description SALSA® MLPA® probemix P067-B3 PTCH1

To be used with the MLPA General Protocol.

P067 Version B3. As compared to version B2, five reference probes have been replaced and one reference probe has been removed. For complete product history see page 6.

Catalogue numbers:
- **P067-025R:** SALSA® MLPA® probemix P067 PTCH1, 25 reactions.
- **P067-050R:** SALSA® MLPA® probemix P067 PTCH1, 50 reactions.
- **P067-100R:** SALSA® MLPA® probemix P067 PTCH1, 100 reactions.

To be used in combination with a SALSA® MLPA® reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General Information: The SALSA MLPA Probemix P067 PTCH1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PTCH1* gene. The *PTCH1* gene (25 exons) spans ~66 kb of genomic DNA and is located on chromosome 9q22.32, ~95 Mb from the p-telomere. Gorlin syndrome or nevoid basal cell carcinoma syndrome is an autosomal dominant disease characterised by developmental abnormalities and a predisposition to cancers. Defects in the *PTCH1* tumour suppressor gene are the cause of Gorlin syndrome. Furthermore, the *PTCH1* gene is the critical gene for 9q22.3 microdeletion, a disorder characterised by delayed development, particularly affecting the development of motor skills such as sitting, standing, and walking.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK61984/

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/

Probemix content: The SALSA MLPA Probemix P067 PTCH1 contains 33 MLPA probes with amplification products between 142 and 454 nt. This P067 probemix contains probes for 23 out of 25 exons of the *PTCH1* gene, no probes are included for exons 1 and 9. In addition, ten reference probes are included in this probemix, detecting ten different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

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

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

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

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

Required specimens: Extracted DNA from peripheral blood, 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: All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of Gorlin syndrome. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change, therefore samples should be validated before use.

Data analysis: Coffalyser.Net software must be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in

or near a GC-rich region or in or near the *PTCH1* gene. 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 (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 in some cases not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in the *PTCH1* gene are small (point) mutations, none of which will not be detected by using SALSA® MLPA® probemix P067 PTCH1.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by one or more than one consecutive probe (Table 2) 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.

Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *PTCH1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA P067-B3 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) reference	*PTCH1*
64-105	Control fragments – see table in probemix content section for more information		
142	Reference probe 17049-L20127	7q31	
148	**PTCH1 probe** 03707-L03161		**Exon 17**
156	**PTCH1 probe** 17281-L20730		**Exon 6**
173	**PTCH1 probe** 02762-L22245		**Exon 2**
184	**PTCH1 probe** 03708-L22246		**Exon 19**
196 *	Reference probe 11429-L12155	1q41	
202	**PTCH1 probe** 03709-L03163		**Exon 20**
211	**PTCH1 probe** 02763-L21161		**Exon 3**
221	**PTCH1 probe** 02764-L06802		**Exon 4**
229	**PTCH1 probe** 03710-L03164		**Exon 22**
238 *	Reference probe 17870-L22129	2p21	
247	**PTCH1 probe** 02765-L02194		**Exon 7**
254	**PTCH1 probe** 03711-L03165		**Exon 23**
263 *	Reference probe 21243-L29765	3p21	
274	**PTCH1 probe** 02766-L02195		**Exon 12**
292	**PTCH1 probe** 03712-L22247		**Exon 24**
301	**PTCH1 probe** 17280-L22248		**Exon 5**
310	**PTCH1 probe** 02767-L22249		**Exon 16**
328	Reference probe 18523-L23814	5q31	
337	**PTCH1 probe** 02768-L22251		**Exon 18**
346	**PTCH1 probe** 04786-L22252		**Exon 11**
355	Reference probe 10086-L10510	8q22	
364	**PTCH1 probe** 02769-L22253		**Exon 21**
373	Reference probe 00546-L01247	11q22	
382	**PTCH1 probe** 02770-L02160		**Exon 25**
391	**PTCH1 probe** 03705-L03159		**Exon 14**
400 *	Reference probe 18070-L22460	16q23	
409	**PTCH1 probe** 03981-L06799		**Exon 10**
418	**PTCH1 probe** 04787-L04162		**Exon 13**
427	**PTCH1 probe** 04788-L04163		**Exon 8**
436 *	Reference probe 17464-L21220	12p13	
445	**PTCH1 probe** 17279-L20728		**Exon 15**
454	Reference probe 13348-L14774	18q21	

* New in version B3 (from lot B3-0418 onwards).

Note: The exon numbering used in this P067-B3 PTCH1 product description is the exon numbering from the RefSeq transcript NM_000264.3, which is identical to the LRG_515 sequence. The exon numbering and NM sequence used is from June 2017, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. *PTCH1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*PTCH1* Exon	Ligation site NM_000264.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start codon	*189-191 (exon 2)*		
	No Probe	*Exon 1*			
173 «	02762-L22245	Exon 2	36-37	GCGGCTGGTCTG-TCAACCGGAGCC	2.1 kb
211 «	02763-L21161	Exon 3	538-539	GGTGGGATTAAA-AGCAGCGAACCT	20.7 kb
221	02764-L06802	Exon 4	676-677	ACAGACCCCTAA-AGAAGAAGGTGC	3.6 kb
301	17280-L22248	Exon 5	806-807	TGTTACAAATCA-GGAGAGCTTATC	0.1 kb
156	17281-L20730	Exon 6	34 nt before exon 6	GTGTGCCTTAAC-CTAACGCATGGC	1.6 kb
247	02765-L02194	Exon 7	1036-1037	GGAAATGCTGAA-TAAGGCTGAGGT	0.5 kb
427	04788-L04163	Exon 8	1187-1188	TTATCCAGAAAG-TATATGCACTGG	1.9 kb
	No Probe	*Exon 9*			
409	03981-L06799	Exon 10	1436-1437	CAGAACTCCACT-CAAAAGGTGCTT	0.6 kb
346	04786-L22252	Exon 11	1638-1639	CAGTGGCTGCAG-GACTGGGCCTGT	0.8 kb
274	02766-L02195	Exon 12	1714-1715	TCTCGCTCTTGG-TGTTGGTGTGGA	0.7 kb
418	04787-L04162	Exon 13	1813-1814	GGAGTGCCTGAA-GCGCACAGGAGC	6.2 kb
391	03705-L03159	Exon 14	1959-1960	TGGTTCTGCTCA-TTTTTCCTGCAA	0.8 kb
445	17279-L20728	Exon 15	2071-2072	GGTTGAACCTCA-GGCCTACACCGA	1.8 kb
310	02767-L22249	Exon 16	2522-2523	CTGGACCTTACG-GACATTGTACCT	5.4 kb
148	03707-L03161	Exon 17	2799-2800	AAATCATGCCAA-ACAATTACAAGA	2.2 kb
337	02768-L22251	Exon 18	2926-2927	TGCAGATGGCAT-CATTAATCCCAG	1.5 kb
184	03708-L22246	Exon 19	3165-3166	AGGCAATTGAAA-AAGTAAGGACCA	1.8 kb
202	03709-L03163	Exon 20	3398-3399	CTGTTCGGCATG-ATGGGCCTCATC	2.8 kb
364	02769-L22253	Exon 21	3516-3517	CGGCCATCGGCG-ACAAGAACCGCA	3.7 kb
229	03710-L03164	Exon 22	3695-3696	AATGGGCTGGTT-TTGCTTCCCGTG	0.7 kb
254	03711-L03165	Exon 23	3865-3866	CTCGGAGTATAG-TTCCCAGACGAC	1.9 kb
292	03712-L22247	Exon 24	4157-4158	AGAGACGCTTTT-GAAATTTCTACT	2.2 kb
382	02770-L02160	Exon 25	5873-5874	GGGGATTCTTCA-TGCACCAGTGTT	
		Stop codon	*4530-4532 (exon 24)*		

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Note: The exon numbering used in this P067-B3 PTCH1 product description is the exon numbering from the RefSeq transcript NM_000264.3, which is identical to the LRG_515 sequence. The exon numbering and NM sequence used is from June 2017, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

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 P067 PTCH1

- Bholah et al. 2014. Germline Mutations in SUFU Cause Gorlin Syndrome–Associated Childhood Medulloblastoma and Redefine the Risk Associated With PTCH1 Mutations. *J Clin Oncol*. 32:4155-61.
- Smith et al. 2014. Germline Mutations in SUFU Cause Gorlin Syndrome–Associated Childhood Medulloblastoma and Redefine the Risk Associated With PTCH1 Mutations. *J Clin Oncol*. 32:4155-61.
- Garavelli et al. 2013. Multiple tumor types including leiomyoma and Wilms tumor in a patient with Gorlin syndrome due to 9q22.3 microdeletion encompassing the PTCH1 and FANC-C loci. *Am J Med Genet A*. 161A:2894-901.
- Aradhya et al. 2012. Exon-level array CGH in a large clinical cohort demonstrates increased sensitivity of diagnostic testing for Mendelian disorders. *Genet Med.* 14:594-603.
- Pastorino et al. 2009. Identification of a SUFU germline mutation in a family with Gorlin syndrome. *Am J Med Genet A*. 149A:1539-43.
- Soufir et al. 2006. *PTCH* mutations and deletions in patients with typical nevoid basal cell carcinoma syndrome and in patients with a suspected genetic predisposition to basal cell carcinoma: a French study. *Br J of Canc.* volume 95, pages 548–553.

P067 Product history	
Version	*Modification*
B3	Five reference probes have been replaced and one reference probe has been removed.
B2	One reference probe has been replaced.
B1	One probe for PTCH1 exon 8 has been added, the probe for exon 12 removed and exon 9 and 18 replaced. Flanking probe FANCC has been removed. One ref has been removed, eight replaced.
A2	QDX2 control fragments included
A1	First release.

Implemented changes in the product description

Version B3-01 – 30 May 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed. lot number added. small changes in Table 1 and Table 2. new picture included).

Version 14 (54)- 27 January 2015
- Product description adapted to a new lot (lot number added, exon numbering changed, small changes in Table 1, new picture included).
- Various minor textual changes.

Version 13 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 12 (48)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes.

Version 11 (48)
- Remark on RefSeqGene standard and transcript variant added below Table 2.
- References added on page 1.
- Various minor textual changes.

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