Product Description
SALSA® MLPA® Probemix P067 PTCH1

To be used with the MLPA General Protocol.

Version B3
For complete product history see page 7.

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 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 P067 PTCH1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PTCH1* gene, which is associated with Gorlin syndrome or nevoid basal cell carcinoma syndrome (NBCCS) and 9q22.3 microdeletion.

Gorlin syndrome/NBCCS is an autosomal dominant disease characterised by developmental abnormalities and a predisposition to cancers. Defects in the *PTCH1* tumour suppressor gene (25 exons, spans ~66 kb of genomic DNA and is located on chromosome 9q22.32, ~95 Mb from the p-telomere) 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/NBK1151/ and 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/

Exon numbering
The *PTCH1* exon numbering used in this P067-B3 PTCH1 product description is the exon numbering from the LRG_515 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 LRG 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 P067-B3 PTCH1 contains 33 MLPA probes with amplification products between 142 and 454 nucleotides (nt). This includes 23 probes for the *PTCH1* gene, one probe for each exon with the exception of exons 1 and 9. In addition, ten 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 Gorlin syndrome/NBCCS and 9q22.3 microdeletion. 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 *PTCH1* gene are small (point) mutations, none of which will 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. 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.

***PTCH1* mutation database**

https://databases.lovd.nl/shared/genes/PTCH1. 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 *PTCH1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P067-B3 PTCH1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*PTCH1*
64-105	Control fragments – see table in probemix content section for more information		
142	Reference probe 17049-L20127	7q	
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	1q	
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	2p	
247	**PTCH1 probe** 02765-L02194		**Exon 7**
254	**PTCH1 probe** 03711-L03165		**Exon 23**
263	Reference probe 21243-L29765	3p	
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	5q	
337	**PTCH1 probe** 02768-L22251		**Exon 18**
346	**PTCH1 probe** 04786-L22252		**Exon 11**
355	Reference probe 10086-L10510	8q	
364	**PTCH1 probe** 02769-L22253		**Exon 21**
373	Reference probe 00546-L01247	11q	
382	**PTCH1 probe** 02770-L02160		**Exon 25**
393	**PTCH1 probe** 03705-L03159		**Exon 14**
400	Reference probe 18070-L22460	16q	
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	12p	
445	**PTCH1 probe** 17279-L20728		**Exon 15**
454	Reference probe 13348-L14774	18q	

[a] See section Exon numbering on page 1 for more information. Exon 1 is only present in transcript variant 2 (NM_001083602.1).

« 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.

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. *PTCH1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*PTCH1* exon[a]	Ligation site NM_000264.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*906-908 (Exon 2)*		
	No probe	*Exon 1*			
173 «	02762-L22245	Exon 2	753-754	GCGGCTGGTCTG-TCAACCGGAGCC	2.1 kb
211 «	02763-L21161	Exon 3	1255-1256	GGTGGGATTAAA-AGCAGCGAACCT	20.7 kb
221	02764-L06802	Exon 4	1393-1394	ACAGACCCCTAA-AGAAGAAGGTGC	3.6 kb
301	17280-L22248	Exon 5	1523-1524	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	1753-1754	GGAAATGCTGAA-TAAGGCTGAGGT	0.5 kb
427	04788-L04163	Exon 8	1904-1905	TTATCCAGAAAG-TATATGCACTGG	1.9 kb
	No probe	*Exon 9*			
409	03981-L06799	Exon 10	2153-2154	CAGAACTCCACT-CAAAAGGTGCTT	0.6 kb
346	04786-L22252	Exon 11	2355-2356	CAGTGGCTGCAG-GACTGGGCCTGT	0.8 kb
274	02766-L02195	Exon 12	2431-2432	TCTCGCTCTTGG-TGTTGGTGTGGA	0.7 kb
418	04787-L04162	Exon 13	2530-2531	GGAGTGCCTGAA-GCGCACAGGAGC	6.2 kb
393	03705-L03159	Exon 14	2676-2677	TGGTTCTGCTCA-TTTTTCCTGCAA	0.8 kb
445	17279-L20728	Exon 15	2788-2789	GGTTGAACCTCA-GGCCTACACCGA	1.8 kb
310	02767-L22249	Exon 16	3239-3240	CTGGACCTTACG-GACATTGTACCT	5.4 kb
148	03707-L03161	Exon 17	3516-3517	AAATCATGCCAA-ACAATTACAAGA	2.2 kb
337	02768-L22251	Exon 18	3643-3644	TGCAGATGGCAT-CATTAATCCCAG	1.5 kb
184	03708-L22246	Exon 19	3882-3883	AGGCAATTGAAA-AAGTAAGGACCA	1.8 kb
202	03709-L03163	Exon 20	4115-4116	CTGTTCGGCATG-ATGGGCCTCATC	2.8 kb
364	02769-L22253	Exon 21	4233-4234	CGGCCATCGGCG-ACAAGAACCGCA	3.7 kb
229	03710-L03164	Exon 22	4412-4413	AATGGGCTGGTT-TTGCTTCCCGTG	0.7 kb
254	03711-L03165	Exon 23	4582-4583	CTCGGAGTATAG-TTCCCAGACGAC	1.9 kb
292	03712-L22247	Exon 24	4874-4875	AGAGACGCTTTT-GAAATTTCTACT	2.2 kb
382	02770-L02160	Exon 25	6590-6591	GGGGATTCTTCA-TGCACCAGTGTT	
		stop codon	*5247-5249 (Exon 24)*		

[a] See section Exon numbering on page 1 for more information. Exon 1 is only present in transcript variant 2 (NM_001083602.1).

[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.

« 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.

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

P158 JPS	Contains probes for *BMPR1A*, *SMAD4*, and *PTEN* genes, involved in juvenile polyposis.
P187 Holoprosencephaly	Contains probes for the *SHH*, *ZIC2*, *SIX3*, *TGIF1*, *TRAPPC10*, *GLI2*, *PTCH1*, and *FBXW11* genes, involved in holoprosencephaly (HPE).
P225 PTEN	Contains probes for the *PTEN* gene, involved in familial meningioma.
P472 SUFU	Contains probes for the *SUFU* gene, involved in familial medulloblastoma and meningioma.

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

- 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.
- 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.
- Kim B et al. (2021). Clinical and genetic profiling of nevoid basal cell carcinoma syndrome in Korean patients by whole-exome sequencing. *Sci Rep*, 11(1), 1-7.
- Martinez MF et al. (2019). Nevoid basal cell carcinoma syndrome: PTCH1 mutation profile and expression of genes involved in the hedgehog pathway in Argentinian patients. *Cells*, 8(2), 144.
- Maturo MG et al. (2020). Coding and noncoding somatic mutations in candidate genes in basal cell carcinoma. *Sci Rep*, 10(1), 1-10.
- Ozcan G et al. (2016). A novel PTCH1 gene mutation in a pediatric patient associated multiple keratocystic odontogenic tumors of the jaws and Gorlin–Goltz syndrome. *Indian J pathol microbiol*, 59(3), 335.
- Smith MJ & Evans DG (2022). PTCH2 is not a strong candidate gene for Gorlin syndrome predisposition. *Fam Cancer*, 21(3), 343-346.
- Smith MJ et al. (2016). The contribution of whole gene deletions and large rearrangements to the mutation spectrum in inherited tumor predisposing syndromes. *Hum mutat*, 37(3), 250-256.

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 B3-02 – 30 November 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *PTCH1* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Related SALSA MLPA probemixes section has been included.
- Selected publication list has been updated.

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).

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200