# Product Description

# SALSA® MLPA® Probemix P071-B2 LMNB1-PLP1-NOTCH3

To be used with the MLPA General Protocol.

**Version B2**
For complete product history see page 7.

**Catalogue numbers:**

- **P071-025R:** SALSA MLPA Probemix P071 LMNB1-PLP1-NOTCH3, 25 reactions.
- **P071-050R:** SALSA MLPA Probemix P071 LMNB1-PLP1-NOTCH3, 50 reactions.
- **P071-100R:** SALSA MLPA Probemix P071 LMNB1-PLP1-NOTCH3, 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 P071 LMNB1-PLP1-NOTCH3 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *LMNB1*, *PLP1* and *NOTCH3* genes, which are associated with autosomal dominant leukodystrophy with autonomic disease (ADLD), Pelizaeus-Merzbacher disease (PMD), and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), respectively.

Autosomal dominant leukodystrophy with autonomic disease (ADLD) is a slowly progressive neurological disorder that is characterized by widespread loss of myelin in the central nervous system. Symptoms develop in the fourth or fifth decade of life and include problems with the autonomous nervous system, followed by movement difficulties. ADLD is caused by mutations in the *LMNB1* gene on chromosome 5q23.2, with most cases resulting from a duplication of the *LMNB1* gene.

Pelizaeus-Merzbacher disease (PMD) is a rare X-linked neurological disorder that is caused by demyelination in the central nervous system. The age of onset is typically during infancy or early childhood. PMD is caused by mutations in the *PLP1* gene on chromosome Xq22.2. Duplication of the complete *PLP1* gene accounts for 50-70% of PMD cases, whereas point mutations and deletions are involved in the majority of the remaining cases. In addition to PMD, defects in the *PLP1* gene can also result in spastic paraplegia type 2 (SPG2).

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a condition characterized by migraine, strokes, mood disorders, and cognitive decline that can result in dementia. CADASIL has a mid-adult onset and is caused by mutations in the *NOTCH3* gene on chromosome 19p13.12. Defects in the *NOTCH3* gene can also lead to lateral meningocele syndrome.

**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 *LMNB1, PLP1 and NOTCH3* exon numbering used in this P071-B2 LMNB1-PLP1-NOTCH3 product description is the exon numbering from the NG_008360.2, NG_016452.2 and NG_009819.1 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 P071-B2 LMNB1-PLP1-NOTCH3 contains 34 MLPA probes with amplification products between 122 and 415 nucleotides. This includes 12 probes for the *LMNB1* gene, one probe for each exon and two probes for exon 1. It also includes seven probes for the *PLP1* gene, one probe for each exon with the exception of exon 1, and three probes for the *NOTCH3* gene, targeting exons 2, 7 and 28. Three flanking probes are included that target regions centromeric and telomeric of the *PLP1* gene. In addition, nine 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 autosomal dominant leukodystrophy with autonomic disease (ADLD), Pelizaeus-

Merzbacher disease (PMD), and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). It is recommended to use samples of the same sex to facilitate interpretation. 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 when **reference samples of the same sex** have been used:

| Copy number status | | Final ratio (FR) |
|---|---|---|
| **Autosomal sequences and X chromosome sequences in females** | **X chromosome sequences in males** | |
| Normal | Normal | 0.80 < FR < 1.20 |
| Homozygous deletion | Deletion | FR = 0 |
| Heterozygous deletion | | 0.40 < FR < 0.65 |
| Heterozygous duplication | | 1.30 < FR < 1.65 |
| Heterozygous triplication/homozygous duplication | 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 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 *LMNB1*, *PLP1* and *NOTCH3* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P071-B2 LMNB1-PLP1-NOTCH3.
- 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.

***LMNB1*, *PLP1* and *NOTCH3* mutation databases:**

https://databases.lovd.nl/shared/genes/LMNB1, https://databases.lovd.nl/shared/genes/PLP1, https://databases.lovd.nl/shared/genes/NOTCH3. We strongly encourage users to deposit positive results in the Malacards Human Disease 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 *LMNB1* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P071-B2 LMNB1-PLP1-NOTCH3

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | |
|---|---|---|---|---|---|
| | | Reference | *LMNB1* | *NOTCH3* | *PLP1* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | |
| 122 | Reference probe 02844-L02274 | 18q | | | |
| 129 ¬ | AR probe 01690-L00423 | | | | Xq12 |
| 136 | Reference probe 07292-L06929 | 6q | | | |
| 142 | **PLP1 probe** 09208-L11144 | | | | **Exon 5** |
| 151 | **PLP1 probe** 01274-L11199 | | | | **Exon 2** |
| 157 | **PLP1 probe** 09209-L00828 | | | | **Exon 6** |
| 165 | Reference probe 08357-L08210 | 17q | | | |
| 171 | Reference probe 16243-L18501 | 11p | | | |
| 177 | **LMNB1 probe** 09221-L09539 | | **Exon 5** | | |
| 184 | **LMNB1 probe** 09224-L09542 | | **Exon 8** | | |
| 197 | **LMNB1 probe** 09220-L10868 | | **Exon 4** | | |
| 206 | **NOTCH3 probe** 09228-L10869 | | | **Exon 2** | |
| 214 | **LMNB1 probe** 09223-L09541 | | **Exon 7** | | |
| 220 | **NOTCH3 probe** 09232-L11200 | | | **Exon 28** | |
| 226 | **LMNB1 probe** 09217-L09535 | | **Exon 1** | | |
| 232 | **PLP1 probe** 01276-L31608 | | | | **Exon 3** |
| 239 ¬ | FRMD7 probe 08248-L22041 | | | | Xq26 |
| 246 | **LMNB1 probe** 09222-L09540 | | **Exon 6** | | |
| 256 | **LMNB1 probe** 09225-L09543 | | **Exon 9** | | |
| 264 | **PLP1 probe** 01278-L11204 | | | | **Exon 4** |
| 268 | **LMNB1 probe** 09218-L11205 | | **Exon 2** | | |
| 283 | Reference probe 09101-L09160 | 4q | | | |
| 292 | **LMNB1 probe** 09226-L09544 | | **Exon 10** | | |
| 301 | **LMNB1 probe** 09216-L09534 | | **Exon 1** | | |
| 310 | **NOTCH3 probe** 09229-L09547 | | | **Exon 7** | |
| 317 | **LMNB1 probe** 09219-L09537 | | **Exon 3** | | |
| 328 | **LMNB1 probe** 09227-L09545 | | **Exon 11** | | |
| 337 | Reference probe 09937-L12248 | 8q | | | |
| 346 | Reference probe 07207-L06857 | 7p | | | |
| 355 | Reference probe 07236-L06886 | 1q | | | |
| 364 | **PLP1 probe** 09185-L00830 | | | | **Exon 8** |
| 373 | **PLP1 probe** 01282-L00965 | | | | **Exon 7** |
| 385 ¬ | PAK3 probe 02908-L03178 | | | | Xq23 |
| 415 | Reference probe 07594-L29165 | 15q | | | |

[a] See section Exon numbering on page 2 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.

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. P071-B2 probes arranged according to chromosomal location

Table 2a. *LMNB1*

| Length (nt) | SALSA MLPA probe | *LMNB1* exon[a] | Ligation site NM_005573.4 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *Start Codon* | *374-376 (Exon 1)* | | |
| 301 | 09216-L09534 | Exon 1 | 35-36 | CAAAGTGCTGCG-AGCAGGAGACGG | 0.7 kb |
| 226 | 09217-L09535 | Exon 1 | 687-688 | CAAGCTGCAGAT-CGAGCTGGGCAA | 27.1 kb |
| 268 | 09218-L11205 | Exon 2 | 836-837 | CTGCACTTGGTG-ACAAAAAAAGTT | 0.7 kb |
| 317 | 09219-L09537 | Exon 3 | 925-926 | AAAAAACAGTTA-GCAGATGAAACT | 4.6 kb |
| 197 | 09220-L10868 | Exon 4 | 1058-1059 | CGCGCTTGGTAG-AGGTGGATTCTG | 1.6 kb |
| 177 | 09221-L09539 | Exon 5 | 1225-1226 | ATGAATACTTCT-ACTGTCAACAGT | 7.2 kb |
| 246 | 09222-L09540 | Exon 6 | 1403-1404 | CAGACAAAGAGA-GAGAGATGGCGG | 2.0 kb |
| 214 | 09223-L09541 | Exon 7 | 1657-1658 | GAGGCGAGTAGT-AGTGTTAGCATC | 1.8 kb |
| 184 | 09224-L09542 | Exon 8 | 1831-1832 | AAATATACCTCA-AGATATGTGCTG | 3.2 kb |
| 256 | 09225-L09543 | Exon 9 | 1963-1964 | GTGAAGGTTATA-TTGAAAAATTCT | 6.7 kb |
| 292 | 09226-L09544 | Exon 10 | 2046-2047 | GGAGGAGGAGGA-AGAAGCAGCTGG | 4.1 kb |
| 328 | 09227-L09545 | Exon 11 | 2695-2696 | AGCACTCTGGAT-GATGGATTCCAC | |
| | | *Stop Codon* | *2132-2134 (Exon 11)* | | |

Table 2b. *PLP1*

| Length (nt) | SALSA MLPA probe | *PLP1* exon[a] | Ligation site NM_001128834.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 129 ¬ | 01690-L00423 | *AR* | | CATGCAACTCCT-TCAGCAACAGCA | 36.2 **M**b |
| | | | | | |
| | | *Start Codon* | *286-288 (Exon 2)* | | |
| | *No probe* | *Exon 1* | | | |
| 151 | 01274-L11199 | Exon 2 | 243-244 | AGCCACAAAGCA-GACTAGCCAGCC | 8.7 kb |
| 232 | 01276-L31608 | Exon 3 | 360-361 | ACTGGATTGTGT-TTCTTTGGGGTG | 1.0 kb |
| 264 | 01278-L11204 | Exon 4 | 684-685 | CATCAAGCTCAT-TCTTTGGAGCGG | 1.2 kb |
| 142 | 09208-L11144 | Exon 5 | 811-812 | TGCCTGTGTACA-TTTACTTCAACA | 0.6 kb |
| 157 | 09209-L00828 | Exon 6 | 939-940 | TTCCCTGGCAAG-GTTTGTGGCTCC | 0.9 kb |
| 373 | 01282-L00965 | Exon 7 | 1013-1014 | GTTTATTGCTGC-ATTTGTGGGGGC | 1.2 kb |
| 364 | 09185-L00830 | Exon 8 | 1077-1078 | GCCACTTACAAC-TTTGCCGTCCTT | 7.3 **M**b |
| | | *Stop Codon* | *1117-1119 (Exon 8)* | | |
| | | | | | |
| 385 ¬ | 02908-L03178 | *PAK3* | | CGGGATTCTTCA-GCACTCAACCAC | 20.8 **M**b |
| 239 ¬ | 08248-L22041 | *FRMD7* | | ATTCTGCAGCCA-TTCTGGAAATAA | |

Table 2c. *NOTCH3*

| Length (nt) | SALSA MLPA probe | *NOTCH3* exon[a] | Ligation site NM_000435.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *Start Codon* | *91-93 (Exon 1)* | | |
| 206 | 09228-L10869 | Exon 2 | 252-253 | AATGGAGGTCGT-TGCACCCAGCTG | 8.1 kb |
| 310 | 09229-L09547 | Exon 7 | 1155-1156 | GATGACGCCTGT-GTCAGCAACCCC | 19.3 kb |
| 220 | 09232-L11200 | Exon 28 | 5257-5258 | CAGACTGGATGG-ACACAGAGTGCC | |
| | | *Stop Codon* | *7054-7056 (Exon 33)* | | |

[a] See section Exon numbering on page 2 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.

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.

Complete probe sequences are available at www.mrcholland.com.

## Related SALSA MLPA probemixes

P022 PLP1 Contains probes for the *PLP1* gene, involved in Pelizaeus-Merzbacher disease.

## References


- Gong Y et al. (2001). LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell*, 107(4). 513-523.
- 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 P071 LMNB1-PLP1-NOTCH3


- Brussino A et al. (2010). A family with autosomal dominant leukodystrophy linked to 5q23.2-q23.3 without lamin B1 mutations. *Eur J Neurol*. 17:541-549.
- Dai Y et al. (2017). An LMNB1 duplication caused adult-onset autosomal dominant leukodystrophy in Chinese family: clinical manifestations, neuroradiology and genetic diagnosis. *Front Mol Neurosci*. 10:215.
- Sandoval-Rodriguez V et al. (2017). Autosomal dominant leukodystrophy presenting as Alzheimer's-type dementia. *Mult Scler Relat Disord*. 17:230-233.
- Westenberger A et al. (2013). X-linked Dystonia-Parkinsonism manifesting in a female patient due to atypical turner syndrome. *Mov Disord*. 28:675-678.


| **P071 product history** | |
|---|---|
| *Version* | *Modification* |
| B2 | One reference probe and one flanking probe have been removed, and one target probe has been changed in length but not in sequence detected. |
| B1 | Three reference probes have been replaced and one NOTCH3 probe has been removed. |
| A2 | The 118 nt Y-chromosome probe has been removed and the control fragments have been replaced by QDX2 control fragments. |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version B2-02 – 22 September 2022 (04P)<br>- Product description rewritten and adapted to a new template.<br>- Ligation sites of the probes targeting the *PLP1* gene updated according to new version of the NM_ reference sequence.<br>- Salt sensitivity warnings have been removed from table 1 and 2.<br>Version B2-01 — 07 August 2019 (02P)<br>- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).<br>- Product description rewritten and adapted to a new template.<br>- Ligation sites of the probes targeting the *LMNB1* and *NOTCH3* genes updated according to new version of the NM_ reference sequence. |

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