# Product Description
# SALSA® MLPA® Probemix P405-B1 CMT1

To be used with the MLPA General Protocol.

**Version B1**
For complete product history see page 10.

**Catalogue numbers:**
- **P405-025R:** SALSA MLPA Probemix P405 CMT1, 25 reactions.
- **P405-050R:** SALSA MLPA Probemix P405 CMT1, 50 reactions.
- **P405-100R:** SALSA MLPA Probemix P405 CMT1, 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.

**Intended purpose**
The SALSA MLPA Probemix P405 CMT1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the human *PMP22*, *MPZ* and *GJB1* genes in genomic DNA isolated from human peripheral whole blood specimens or buccal swabs. P405 CMT1 is intended to confirm a potential cause for and clinical diagnosis of Charcot-Marie-Tooth disease type 1 (CMT1), X-linked CMT (CMTX) or hereditary neuropathy with liability to pressure palsies (HNPP) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P405 CMT1 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in *MPZ* and *GJB1*, and some defects in *PMP22* are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

**Clinical background**
Charcot-Marie-Tooth disease (CMT), with a worldwide incidence of 1 in 2500, is the most common hereditary sensorimotor neuropathy, comprising a group of clinically and genetically heterogeneous peripheral neuropathies. CMT is characterized by progressive distal muscle atrophy and weakness, sensory disturbance, the absence of deep tendon reflexes, and *pes cavus* deformity of the foot. More than 80 different genes are

associated with CMT (http://www.ncbi.nlm.nih.gov/books/NBK1358/). Subtypes related to the genes *PMP22*, *GJB1*, *MPZ* and *MFN2* are the most common ones, being responsible for up to 95% of CMT cases with a final diagnosis (Padilha et al. 2020). The disease can be inherited in an autosomal dominant, autosomal recessive or X-linked manner. Table 1 provides an overview of the different genes involved in the CMT subtypes and the probemixes that cover these genes.

The most frequent form, CMT1A, accounts for as much as 50% of all CMT cases. CMT1A is a dominantly inherited, childhood-onset, slowly progressive motor and sensory neuropathy due to a duplication of *PMP22* on chromosome 17. CMT1B accounts for an additional ~10% of CMT1 patients. The gene involved in CMT1B is myelin protein zero (*MPZ*). CMT1A and 1B are clinically indistinguishable; classification is based solely on molecular findings.

X-linked CMT (CMTX) is characterized by moderate to severe motor and sensory neuropathy in affected males and usually mild to no symptoms in carrier females. Subtype CMTX1 accounts for about 90% of X-linked CMT, and is caused by mutations in the gap-junction protein beta 1 (*GJB1*) gene (https://www.ncbi.nlm.nih.gov/books/NBK1374/).

Hereditary neuropathy with liability to pressure palsies (HNPP) is characterized by repeated focal pressure neuropathies such as carpal tunnel syndrome and peroneal palsy with foot drop. Recovery from acute neuropathy is often complete; when recovery is not complete, the resulting disability is usually mild. Some affected individuals also have signs of a mild to moderate peripheral neuropathy. The prevalence of HNPP is estimated at 7-16 in 100,000. The penetrance is 100% but expressivity is highly variable even within the same family. Approximately 6-23% of individuals diagnosed with HNPP have an asymptomatic affected parent. A contiguous gene deletion of chromosome 17p12 that includes *PMP22* is present in approximately 80% of affected individuals; the remaining 20% have a pathogenic variant in *PMP22* (https://www.ncbi.nlm.nih.gov/books/NBK1392/).

**Table 1.** Overview of the probemixes and genes related to CMT.

| Probemix* | Genes and coverage | Condition | Remarks |
|---|---|---|---|
| P033-B4<br>CMT1 (IVD) | *PMP22*: all exons<br>*KIF1b*: 2 probes | CMT1A and HNPP<br>CMT2A1 | PMP22 probes in P033-B4 have the same ligation site as *PMP22* probes in P405-B1 except for one exon 1 probe and one exon 4 probe. There is one additional PMP22 exon 5 probe present in P033-B4. |
| P405-B1<br>CMT1 (IVD) | *PMP22*: all exons<br>*MPZ*: all exons<br>*GJB1*: all exons | CMT1A and HNPP<br>CMT1B<br>CMTX | PMP22 probes in P405-B1 have the same ligation sites as PMP22 probes in P033-B4 except for one exon 1 probe and one exon 4 probe. There is one additional PMP22 exon 5 probe present in P033-B4.<br>MPZ probes in P405-B1 have the same ligation sites as MPZ probes in P143-C3. |
| P143-C3<br>MFN2-MPZ<br>(RUO) | *MFN2*: all exons<br>*MPZ*: all exons | CMT2A<br>CMT1B | MPZ probes in P143-C3 have the same ligation sites as MPZ probes in P405-B1. |

*IVD: in vitro diagnostic. RUO: research use only.

**Gene structure**

The *PMP22* gene spans ~35.5 kilobases (kb) on chromosome 17p12 and contains 5 exons. The *PMP22* LRG_263 is available at www.lrg-sequence.org and is identical to GenBank NG_007949.1.

The *MPZ* gene spans ~5.2 kb of genomic DNA on chromosome 1q23.3 and contains 6 exons. The *MPZ* LRG_256 is identical to GenBank NG_008055.1.

The *GJB1* gene spans ~10 kb of genomic DNA on chromosome Xq13.1 and contains 3 exons. The *GJB1* LRG_245 is identical to GenBank NG_008357.1.

**Transcript variants**
For *PMP22,* multiple variants have been described (https://www.ncbi.nlm.nih.gov/gene/5376). Transcript variant 1 encodes isoform 1 (NM_000304.4, 1828 nt, coding sequence 208-690). Alternative splicing results in multiple transcript variants. *PMP22* transcript variants 1-5 all encode isoform 1.

For *MPZ*, two variants have been described (https://www.ncbi.nlm.nih.gov/gene/4359). Transcript variant 1 encodes two isoforms, which result from the use of alternative in-frame translation termination codons. RefSeq transcript NM_000530.8 (1951 nt, coding sequence 64-810) represents the shorter isoform MPZ (also known as P0).

For *GJB1*, two variants have been described (https://www.ncbi.nlm.nih.gov/gene/2705). Transcript variant 1 represents the shorter transcript, and is transcribed from promoter P1 (NM_001097642.3, 1877 nt, coding sequence 69-920). Transcript variant 2 differs in the 5' UTR compared to variant 1, and is transcribed from promoter P2 (NM_000166.6; 1937 nt; coding sequence 129-980). Both variants 1 and 2 encode the same protein.

**Exon numbering**
The *PMP22* and *MPZ* exon numbering used in this P405-B1 CMT1 product description is the exon numbering from the LRG_263 and LRG_256 sequences, respectively. For *GJB1*, the exon numbering from the RefSeq transcript NM_001097642.3 is used. 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 P405-B1 CMT1 contains 42 MLPA probes with amplification products between 130 and 445 nucleotides (nt). This includes 15 probes for the common 17p12 deletion/duplication region, two flanking probes for genes outside the common 17p12 deletion/duplication region, seven probes for the *MPZ* gene, and five probes for the *GJB1* gene. In addition, 10 ten reference probes are included that detect autosomal chromosomal locations and three probes detecting locations on the X-chromosome. 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 of the same sex 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 from human peripheral whole blood specimens or buccal swabs, 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 CMT or HNPP. 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. Sample ID numbers NA05167 and NA12214 from the Coriell Institute have been tested with this P405-B1 probemix at MRC Holland and can be used as a positive control samples to detect a heterozygous duplication of chromosome 17p12 that includes the *PMP22*, *COX10* and *TEKT3* genes. The quality of cell lines can change; therefore samples should be validated before use.

**Performance characteristics**
*PMP22* duplications explain 50% of all CMT cases (https://www.ncbi.nlm.nih.gov/books/NBK1358/) and *PMP22* deletions explain 80% of all HNPP cases (https://www.ncbi.nlm.nih.gov/books/NBK1392/). *GJB1* deletions explain 0.3% of CMT cases (DiVincenzo et al. 2014). No duplications in *GJB1* have been described. No deletions or duplications in *MPZ* have been described in CMT cases, however, the association between *MPZ* mutations and CMT is well established (DiVincenzo et al. 2014). The analytical sensitivity and specificity for the detection of deletions or duplications in *PMP22*, *MPZ* and *GJB1* is very high and can be considered >99% (based on a 2006-2022 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

**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 expected results for the probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). The same results can be expected for the X-chromosome-specific probes in female samples. In rare cases, copy numbers of 0 (homozygous deletion) or

4 (heterozygous triplication/homozygous duplication) may be obtained. For the X-chromosome-specific probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (duplication).

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 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.

P405 specific notes:

- Several types and subtypes of Charcot-Marie-Tooth disease exist, each involving a different gene or locus. Furthermore, there is a large overlap in clinical phenotype between the different types of CMT. Other disorders with comparable clinical features have been described, including hereditary neuralgic amyotrophy (HNA), amyloid neuropathies, Krabbe disease and hereditary ataxias. Therefore, molecular diagnosis may be complicated for certain patients.
- Please note that recurrent duplications have been described which can be detected by the two TEKT3 probes, but not by any of the PMP22 probes. These duplications may cause CMT through an unknown mechanism affecting *PMP22* expression (Weterman et al. 2010; Zhang et al. 2010).

**Limitations of the procedure**

- In most populations, the major cause of genetic defects in the *MPZ* and *GJB1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P405 CMT1.
- 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/PMP22 and https://databases.lovd.nl/shared/genes/MPZ and https://databases.lovd.nl/shared/genes/GJB1. We strongly encourage users to deposit positive results in the 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 *PMP22* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

## Table 2. SALSA MLPA Probemix P405-B1 CMT1

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | |
|---|---|---|---|---|---|
| | | Reference | *MPZ* | *PMP22* / 17p12 | *GJB1* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | |
| 130 | Reference probe 00797-L13645 | 5q | | | |
| 136 | **GJB1 probe** 02629-L02096 | | | | **Exon 2** |
| 142 | **PMP22 probe** 04656-L04039 | | | **Exon 1** | |
| 148 | **PMP22 probe** 04657-L04461 | | | **Exon 3** | |
| 154 | **TEKT3 probe** 22647-L32049 | | | 17p12, CMT1 region | |
| 160 | Reference probe 07394-L07041 | 12q | | | |
| 165 | **MPZ probe** 04895-L31858 | | **Exon 1** | | |
| 172 | **PMP22 probe** 11539-L04463 | | | **Exon 4** | |
| 178 | **MPZ probe** 04896-L04280 | | **Exon 2** | | |
| 184 | **PMP22 probe** 02678-L02158 | | | **Upstream *PMP22*** | |
| 190 | Reference probe 06148-L04992 | 7p | | | |
| 196 | **GJB1 probe** 13202-L14523 | | | | **Exon 1** |
| 203 | **PMP22 probe** 22645-L31860 | | | **Exon 3** | |
| 208 | **MPZ probe** 04897-L32050 | | **Exon 3** | | |
| 213 | **GJB1 probe** 13203-L19630 | | | | **Exon 2** |
| 220 | Reference probe 14968-L16704 | 6q | | | |
| 226 | **MPZ probe** 06139-L05583 | | **Exon 1** | | |
| 232 | **MPZ probe** 04898-L17028 | | **Exon 4** | | |
| 238 | **PMP22 probe** 22648-L31863 | | | **Exon 1** | |
| 244 | **PMP22 probe** 04659-L19632 | | | **Exon 5** | |
| 250 | Reference probe 18056-L31125 | 16q | | | |
| 256 | **PMP22 probe** 01462-L00927 | | | **Exon 2** | |
| 270 | **MPZ probe** 04899-L20744 | | **Exon 5** | | |
| 278 ¬ | DRC3 probe 01452-L20745 | | | 17p11, outside CMT1 region | |
| 286 | Reference probe 07737-L21372 | 20q | | | |
| 293 | **TEKT3 probe** 22649-L32051 | | | 17p12, CMT1 region | |
| 301 | **MPZ probe** 04900-L04284 | | **Exon 6** | | |
| 311 | **PMP22 probe** 22650-L31865 | | | **Exon 4** | |
| 319 | Reference probe 15385-L17792 | 3p | | | |
| 328 ∫ | X chromosome 13522-L14327 | | | | Xp11 |
| 346 ¬ | ELAC2 probe 01466-L00917 | | | 17p12, outside CMT1 region | |
| 355 | **PMP22 probe** 22651-L31866 | | | **Upstream *PMP22*** | |
| 364 | Reference probe 05953-L05397 | 2p | | | |
| 373 | **PMP22 probe** 02729-L02156 | | | **Upstream *PMP22*** | |
| 384 ∫ | X chromosome 13750-L15237 | | | | Xp22 |
| 391 | **GJB1 probe** 22646-L31861 | | | | **Exon 2** |
| 400 | **COX10 probe** 01468-L19633 | | | 17p12, CMT1 region | |
| 409 | Reference probe 02718-L00732 | 14q | | | |
| 418 ∫ | X chromosome 00820-L20737 | | | | Xq25 |
| 427 | **COX10 probe** 01469-L20736 | | | 17p12, CMT1 region | |
| 436 | **GJB1 probe** 06188-L20735 | | | | **Exon 2** |
| 445 | Reference probe 12526-L13576 | 4q | | | |

[a] See section Exon numbering on page 3 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.
∫ X-chromosome probe. Used for the determination of X-chromosome copy number.

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 3. P405-B1 probes arranged according to chromosomal location

Table 3a. *MPZ* gene

| Length (nt) | SALSA MLPA probe | *MPZ* exon[a] | Ligation site NM_000530.8 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *stop codon* | *808-810 (Exon 6)* | | |
| 301 | 04900-L04284 | Exon 6 | 733-734 | ATGCAATGCTGG-ACCACAGCAGAA | 0.2 kb |
| 270 | 04899-L20744 | Exon 5 | 678-679 | TTGCACAAGCCA-GGAAAGGACGCG | 0.3 kb |
| 232 | 04898-L17028 | Exon 4 | 536-537 | CGGGGTCGTTCT-GGGAGCTGTGAT | 0.4 kb |
| 208 | 04897-L32050 | Exon 3 | 386-387 | CCCTCGCTGGAA-GGATGGCTCCAT | 0.5 kb |
| 178 | 04896-L04280 | Exon 2 | 217-218 | TGCACTGCTCCT-TCTGGTCCAGTG | 2.6 kb |
| 165 | 04895-L31858 | Exon 1 | 88-89 | CTCCCTCATCCA-GCCCCAGCCCTA | 0.2 kb |
| | | *start codon* | *64-66 (Exon 1)* | | |
| 226 | 06139-L05583 | Exon 1 | 85 nt before exon 1 | CTGCACATGCCA-GGCTGCAATTGG | |

Table 3b. *PMP22* and 17p12 region

| Length (nt) | SALSA MLPA probe | Gene/exon[a] | Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 346 ¬ | 01466-L00917 | *ELAC2* | | TTGGTCCTGAAT-GAGAACTGTGCC | 1.2 **M**b |
| *Start of common chromosome 17p12 deletion/duplication region* | | | | | |
| 427 | 01469-L20736 | *COX10* | NM_001303.4; 1070-1071 | CTCCTGGCAGTT-TCCTCATTTCAA | 0.2 kb |
| 400 | 01468-L19633 | *COX10* | NM_001303.4; 1273-1274 | CCATCAATGCGT-ACATCTCCTACC | 1.0 **M**b |
| | | | | | |
| | | ***PMP22*** | **NM_000304.4** | | |
| | | *stop codon* | *688-690 (Exon 5)* | | |
| 244 | 04659-L19632 | Exon 5 | 672-673 | ATCTATGTGATC-TTGCGGAAACGC | 8.6 kb |
| 311 | 22650-L31865 | Exon 4 | 454-455 | CTCTGTTCCTGT-TCTTCTGCCAAC | 0.1 kb |
| 172 | 11539-L04463 | Exon 4 | 401-400 reverse | TGGTGGCCTGGA-CAGACTGCAGCC | 19.5 kb |
| 203 | 22645-L31860 | Exon 3 | 367-368 | TCCACCACTGTT-TCTCATCATCAC | 0.1 kb |
| 148 | 04657-L04461 | Exon 3 | 310-309 reverse | GAGATCAGTTGC-GTGTCCATTGCC | 1.5 kb |
| 256 | 01462-L00927 | Exon 2 | 229-230 | TGTTGCTGAGTA-TCATCGTCCTCC | 4.5 kb |
| | | *start codon* | *208-210 (Exon 2)* | | |
| 238 | 22648-L31863 | Exon 1 | 145-146 | AGAAATCTGCTT-GGAAGAAGGGGT | 0.1 kb |
| 142 | 04656-L04039 | Exon 1 | 25-26 | ACCACCAGGGAA-CATCTCGGGGAG | 2.3 kb |
| 355 | 22651-L31866 | Upstream | 2.2 kb before exon 1 | GGTGCTAGAAAT-AGCCAGTCTCAT | 4.2 kb |
| 373 | 02729-L02156 | Upstream | 6.4 kb before exon 1 | GCCTCCATGGTT-AGAGACTAGAAT | 5.9 kb |
| 184 | 02678-L02158 | Upstream | 12.4 kb before exon 1 | TGAAGAGCCCTT-GGATACGGAAGG | 26.4 kb |
| | | | | | |
| 154 | 22647-L32049 | *TEKT3* | NM_031898.3; 1440-1441 | ACACCATCCAGA-CCCTGCAGCAGC | 27.4 kb |
| 293 | 22649-L32051 | *TEKT3* | NM_031898.3; 267-268 | CCCACTCCAATT-TGACCCATAGCC | 2.6 **M**b |
| *End of common chromosome 17p12 deletion/duplication region* | | | | | |
| 278 ¬ | 01452-L20745 | *DRC3* | | CGGATCTCCAAG-ATCGACTCCCTG | |

Table 3c. *GJB1*

| Length (nt) | SALSA MLPA probe | *GJB1* exon[a] | Ligation site NM_001097642.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 196 | 13202-L14523 | Exon 1 | 307 nt after exon 1 reverse | ACTTGGCCACCT-TGCACCTAGGGG | 7.6 kb |
| 391 + | 22646-L31861 | Exon 2 | 483 nt before exon 2 | TAAAGCAGCATA-TGACTCCCCAGC | 0.5 kb |
| | | *start codon* | *69-71 (Exon 2)* | | |
| 136 | 02629-L02096 | Exon 2 | 86-87 | TGGACAGGTTTG-TACACCTTGCTC | 0.4 kb |
| 436 | 06188-L20735 | Exon 2 | 447-448 | ACAAGGTCCACA-TCTCAGGGACAC | 0.7 kb |
| | | *stop codon* | *918-920 (Exon 2)* | | |
| 213 | 13203-L19630 | Exon 2 | 1129-1130 | AGTGCTCAAGGT-TACTGGGAGTGT | |

[a] See section Exon numbering on page 3 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.
+ The ligation site of this probe is located 15 nt before exon 1 of NM_000166.6.

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

| | |
|---|---|
| P033 CMT1 | Contains probes for the 17p12 chromosomal region and *KIF1b* gene. |
| P143 MFN2-MPZ | Contains probes for the *MFN2* and *MPZ* genes. |
| P307 SEPT9 | Contains probes for the *SEPT9* gene involved in hereditary neuralgic amyotrophy. |
| P369 Smith-Magenis | Contains probes for the 17p11.2 Smith-Magenis region. |

## References


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## Selected publications using SALSA MLPA Probemix P405 CMT1


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- Kulshrestha et al. (2017). Deletion of P2 promoter of *GJB1* gene a cause of Charcot-Marie-Tooth disease. *Neuromuscul Disord*. 27:766-770.
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- Nagappa et al. (2020). PMP22 gene–associated neuropathies: Phenotypic spectrum in a cohort from India. *J Mol Neurosc.* 70:778–789.
- Shahrizaila et al. (2014). X-linked Charcot-Marie-Tooth disease predominates in a cohort of multiethnic Malaysian patients. *Muscle Nerve.* 49(2):198-201.
- Wang et al. (2015). Clinical and genetic spectra in a series of Chinese patients with Charcot-Marie-Tooth disease. *Clin Chim Acta.* 451(pt B):263-270.


| **P405 product history** | |
|---|---|
| *Version* | *Modification* |
| B1 | Four reference probes and three target probes have been replaced. |
| A1 | First release. |

**Implemented changes in the product description**

*Version B1-03– 27 January 2023 (04P)*
- Table 1 adjusted; probemix versions added and remarks about PMP22 probes corrected.
- Number of exons in the *GJB1* gene corrected in the Gene structure section.
- Information about transcript variants of *GJB1* updated in the Transcript variants section.
- Source for exon numbering of the *GJB1* gene corrected; exon numbering used is from RefSeq transcript NM_001097642.3 and not from the LRG_245 sequence.
- Chromosomal band of the 418 nt X chromosome probe 00820-L20737 corrected in Table 2.
- Ligation site of the 226 nt MPZ probe 06139-L05583 corrected in Table 3a.
- Remark added to Table 3 about the location of the ligation site of the 391 nt GJB1 probe 22646-L31861.
- Morocco removed as country with IVD status.
- Various minor textual and layout changes.

*Version B1-02– 06 May 2021 (04P)*
- Product description rewritten and adapted to a new template.
- Various minor textual or layout changes.
- Ligation sites of the probes targeting the *GJB1* gene updated according to new version of the NM_ reference sequence.
- UK has been added to the list of countries in Europe that accept the CE mark.

*Version B1-01 — 19 June 2020 (02P)*
- Product description adapted to a new product version (version number changed, changes in Table 2 and Table 3).
- Product description rewritten and adapted to a new template.
- Colombia added as country with IVD status.
- Removed information about P129, this product is discontinued.
- New references added.

*Version A1-03 – 25 April 2019 (04)*
- Product description restructured and adapted to a new template.
- Various minor textual changes.
- Intended use updated.
- Table 1 added to provide an overview of the probemixes and genes related to CMT.
- Ligation sites of the probes targeting the *MPZ*, *PMP22*, *TEKT3* and *COX10* genes updated according to new version of the NM_ reference sequence.

*Version A1-02 – 20 December 2018 (02)*
- Regulatory status section updated to also include Morocco and Israel.

| **More information: www.mrcholland.com; www.mrcholland.eu** | |
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| | |
|---|---|
|  | EUROPE*  <br>ISRAEL<br>COLOMBIA |
|  | ALL OTHER COUNTRIES |

*comprising EU (candidate) member states, members of the European Free Trade Association (EFTA) and the UK. The product is for RUO in all other European countries.