Product Description SALSA® MLPA® Probemix P405-B1 CMT1

To be used with the MLPA General Protocol.

Version B1. As compared to version A1, four reference probes have been replaced and three target probes have been replaced. 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.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.

Intended use: The SALSA MLPA probemix P405 CMT1 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *PMP22*, *MPZ* and *GJB1* genes, in order to confirm a potential cause and clinical diagnosis for Charcot-Marie-Tooth disease type 1 (CMT1). This assay can also be used for the detection of deletions in the human *PMP22* gene, in order to confirm a potential cause and clinical diagnosis for hereditary neuropathy with liability to pressure palsies (HNPP). This assay is for use with human DNA extracted from peripheral blood or saliva. This product can also be used for molecular genetic testing of at-risk family members.

Deletions or duplications obtained with the P405 CMT1 probemix must be confirmed by another technique. In particular, deletions or duplications detected by only a single probe always require validation 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 SALSA MLPA probemix in combination with sequence analysis of the aforementioned genes. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test must be interpreted by a clinical molecular geneticist or equivalent.

[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).

Clinical background: Inherited peripheral neuropathies are among the most common genetic neuromuscular disorders worldwide. The most common form is Charcot-Marie-Tooth (CMT) disease. Prevalence of CMT and related disorders has been estimated to be between 1:2500 and 1:1214. Clinical symptoms of CMT include distal muscle weakness and atrophy, sensory loss, depressed tendon reflexes and high-arched feet (*pes cavus*). At present, more than 80 genes are known to be associated with different types of CMT. The disease can be inherited in an autosomal dominant, autosomal recessive or X-linked manner.

CMT type 1 (CMT1) is a demyelinating peripheral neuropathy which is usually slowly progressive. Several subtypes exist. CMT1A accounts for ~70-80% of all CMT1 cases and this subtype is mainly caused by a ~1.5 Mb duplication on chromosome 17p, including the peripheral myelin protein 22 (*PMP22*) gene and flanking regions. The *de novo* rate of *PMP22* duplications in CMT1 patients is ~10-20%. Furthermore, CMT1A can be caused by activating point mutations in this gene. Increased *PMP22* gene dosage leads to altered nerve conduction velocity, which is the main cause of the clinical manifestations in CMT1A.

Another subtype of CMT disease is hereditary neuropathy with liability to pressure palsies (HNPP), which is characterized by repeated focal pressure neuropathies such as carpal tunnel syndrome and peroneal palsy with foot drop. *PMP22* is the only gene known to be associated with HNPP. 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*. The prevalence of HNPP is estimated at two to five cases per 100.000 individuals. However, the majority of individuals with HNPP probably remain undiagnosed due to the mild phenotype.

CMT type 1B accounts for ~10-12% of all CMT1 cases and is caused by defects in the myelin protein-zero (*MPZ*) gene. MPZ is important in formation and stabilisation of peripheral nerve myelin and interacts with PMP22. Defects in the *MPZ* gene cause progressive slowing of nerve conduction and hypertrophy of Schwann cells. Mutations in *MPZ* can also lead to the more severe polyneuropathies, Dejerine-Sottas syndrome (DSS) and congenital hypomyelinating neuropathy (CHN), as well as several subtypes of CMT2. CMT1A and 1B are clinically indistinguishable; classification is based solely on molecular findings.

X-linked CMT (CMTX) accounts for ~10-15% of all CMT cases and the main subtype, CMTX1, is caused by defects in the *Gap Junction Beta-1* (*GJB1*, also known as *connexin-32*) gene. Carrier females are often asymptomatic, or may experience only mild symptoms. Affected males present with moderate to severe motor and sensory neuropathy. Deafness and central nervous symptoms have been described in some CMTX1 patients. Pathogenic variants in the *GJB1* coding region account for ~90% of CMTX1 cases and deletions of *GJB1* have been documented in rare cases. No duplications of the *GJB1* gene have been reported.

More information on CMT and HNPP can be found on:
Clinical Utility Gene Card: https://www.nature.com/articles/ejhg201075
Gene Reviews: http://www.ncbi.nlm.nih.gov/books/NBK1358/,
https://www.ncbi.nlm.nih.gov/books/NBK1392/, https://www.ncbi.nlm.nih.gov/books/NBK1374/.

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

Probemix	Genes and coverage	Condition	Remarks*
P405	*PMP22* *MPZ* *GJB1*	CMT1A and HNPP CMT1B CMTX1	*PMP22* probes are all identical to P033, except for two probes: one of the exon 1 probes and one of the exon 4 probes detect a different sequence. There is one extra exon 5 probe in P033. *MPZ* probes are all identical to P143.
P033	*PMP22*: all exons *KIF1b*: 2 probes	CMT1A and HNPP CMT2A1	*PMP22* probes are all identical to P405, except for two probes: one of the exon 1 probes and one of the exon 4 probes detect a different sequence. There is one extra exon 5 probe in P033.
P143	*MFN2* *MPZ*	CMT2A CMT1B	*MPZ* probes are all identical to P405.

*Probes are identical in sequence; they can differ in length.

Gene structure: The *PMP22* gene spans ~35.5 kb of genomic DNA on chromosome 17p12 and comprises 5 exons. The *PMP22* LRG_263 sequence 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 comprises 6 exons. The *MPZ* LRG_256 sequence is available at www.lrg-sequence.org and is identical to GenBank NG_008055.1.

The *GJB1* gene spans ~10 kb of genomic DNA on chromosome Xq13.1 and comprises 2 exons. The *GJB1* LRG_245 sequence is available at www.lrg-sequence.org and is identical to GenBank NG_008357.1.

Transcript variants: Five protein coding transcript variants have been described for *PMP22* (http://www.ncbi.nlm.nih.gov/gene/5376). Transcript variant 1 (NM_000304.4, 1828 nt, coding sequence 208-690) is the main transcript. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 2 and the stop codon in exon 5. Transcript variants 2, 3, 4 and 5 differ in the 5' UTR sequence, but encode the same protein as transcript variant 1.

Two protein coding transcript variants have been described for *MPZ* (https://www.ncbi.nlm.nih.gov/gene/4359). Transcript variant 1 (NM_000530.8, 1951 nt, coding sequence 64-810) is the main transcript. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 6.

Two protein coding transcript variants have been described for *GJB1* (https://www.ncbi.nlm.nih.gov/gene/2705). Transcript variant 1 (NM_001097642.2, 1623 nt, coding sequence 96-947) is the main transcript. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site and the stop codon are both located in exon 2. The two transcript variants are transcribed from different promoters, but encode the same protein.

Exon numbering: The exon numbering used in this P405-B1 CMT1 product description is the exon numbering from the RefSeq transcript NM_000304.4 for *PMP22*, NM_000530.8 for *MPZ*, and NM_001097642.2 for *GJB1*. The exon numbering and NM_ sequence used have been retrieved on 05/2020. As changes to the NCBI database can occur after release of this product description, 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 located in the 17p12 region, two flanking probes, seven probes in the *MPZ* gene, and five probes in the *GJB1* gene. In addition, 10 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.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, 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.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 nt and 96 nt fragment 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.mlpa.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 peripheral blood or saliva, 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 unrelated individuals who are from families without a history of Charcot-Marie-Tooth disease. 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.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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 positive control samples to detect a heterozygous duplication on chromosome 17p12, including the *PMP22*, *COX10* and *TEKT3* genes. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics: In approximately two-third of all patients with a CMT phenotype, the molecular cause has been established. More than 90% of CMT cases in which a molecular diagnosis has been reached are associated with changes in four genes (*PMP22*, *GJB1*, *MFN2* and *MPZ*). *PMP22* duplication (a 1.5 Mb duplication at 17p12 that includes *PMP22*) accounts for as much as 50% of all CMT cases. A contiguous gene deletion of chromosome 17p12 that includes *PMP22* is present in approximately 80% of affected HNPP individuals; the remaining 20% have a pathogenic variant in *PMP22*.
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 2006-2019 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) 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.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 expected results for the *PMP22*- and *MPZ*-specific probes in male and female individuals and the *GJB1*-specific probes in female individuals are allele copy numbers of 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication). In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication) may be obtained. For the *GJB1* gene, expected results for male individuals include allele copy numbers of 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 dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Dosage quotient
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < DQ < 1.20
Homozygous deletion	Deletion	DQ = 0
Heterozygous deletion		0.40 < DQ < 0.65
Heterozygous duplication		1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	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. 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Notes on results obtained with the P405 CMT1 probemix:

- 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, most of which will not 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. 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 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.

Inherited Peripheral Neuropathies Mutation Database (IPNMDB): http://www.molgen.ua.ac.be/CMTMutations/Home/Default.cfm. We strongly encourage users to deposit positive results in the IPNMDB database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on www.hgvs.org/mutnomen/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *PMP22* exons 2 and 4 but not exon 3) to MRC-Holland: info@mlpa.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	5q31			
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, within CMT1 region	
160	Reference probe 07394-L07041	12q13			
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	7p14			
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	6q22			
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	16q23			
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	20q13			
293 ¥	**TEKT3 probe** 22649-L32051			17p12, within CMT1 region	
301	**MPZ probe** 04900-L04284		**Exon 6**		
311 *	**PMP22 probe** 22650-L31865			**Exon 4**	
319	Reference probe 15385-L17792	3p22			
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	2p22			
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, within CMT1 region	
409	Reference probe 02718-L00732	14q11			
418 ∫	X chromosome 00820-L20737				Xq26
427	**COX10 probe** 01469-L20736			17p12, within CMT1 region	
436	**GJB1 probe** 06188-L20735				**Exon 2**
445 *	Reference probe 12526-L13576	4q25			

a) See above section on exon numbering for more information.

* New in version B1
¥ Changed in version B1. Minor alteration, no change in sequence detected.
∫ Used for the determination of X-chromosome copy number.
¬ 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.

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	81 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*	NM_018127.7; 1193-1194	TTGGTCCTGAAT-GAGAACTGTGCC	1204.3 kb
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	1023.9 kb
		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	2656.6 kb
End of common chromosome 17p12 deletion/duplication region					
278 ¬	01452-L20745	*DRC3*	NM_031294.4; 610-611	CGGATCTCCAAG-ATCGACTCCCTG	

¬ 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.

Table 3c. *GJB1* gene

Length (nt)	SALSA MLPA probe	*GJB1* exon[a]	Ligation site NM_001097642.2	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	*96-98 (exon 2)*		
136	02629-L02096	Exon 2	113-114	TGGACAGGTTTG-TACACCTTGCTC	0.4 kb
436	06188-L20735	Exon 2	474-475	ACAAGGTCCACA-TCTCAGGGACAC	0.7 kb
		stop codon	*945-947 (exon 2)*		
213	13203-L19630	Exon 2	1156-1157	AGTGCTCAAGGT-TACTGGGAGTGT	

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P033 CMT1: Contains probes for the *PMP22*, *COX10*, *TEKT3* and *KIF1b* genes.
- P143 MFN2-MPZ: Contains probes for the *MFN2* and *MPZ* genes, involved in CMT type 2A and 1B.
- P307 SEPT9: Contains probes for almost all exons of the *SEPT9* gene involved in Hereditary neuralgic amyotrophy (HNA).
- P369 Smith-Magenis syndrome: Contains 21 probes in the 17p11.2 Smith-Magenis region, which is immediately adjacent to the CMT1/HNPP chromosomal region.

References

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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
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P405 Product history	
Version	*Modification*
B1	Four reference probes have been replaced and three target probes have been replaced.
A1	First release.

Implemented changes in the product description

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.

Version A1-01 – 24 June 2016 (02)
- Product description restructured and adapted to a new template.
- Various minor textual and layout changes.
- Ligation sites of the probes targeting the *PMP22* gene updated according to new version of the NM_ reference sequence.
- The name of the *LRRC48* gene (detected by the 278 nt probe) has been changed to *DRC3*.

Version 05 – 05 February 2016 (54)
- GJB1 exon numbering updated.
- Minor textual changes on page 1.

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IVD	EUROPE* CE MOROCCO ISRAEL COLOMBIA
RUO	ALL OTHER COUNTRIES

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