Product Description
SALSA® MLPA® Probemix P033-B4 CMT1

To be used with the MLPA General Protocol.

Version B4
For complete product history see page 10.

Catalogue numbers:
- **P033-025R:** SALSA MLPA Probemix P033 CMT1, 25 reactions.
- **P033-050R:** SALSA MLPA Probemix P033 CMT1, 50 reactions.
- **P033-100R:** SALSA MLPA Probemix P033 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 P033 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* and *KIF1b* genes in genomic DNA isolated from human peripheral whole blood specimens or buccal swabs. P033 CMT1 is intended to confirm a potential cause for and clinical diagnosis of Charcot-Marie-Tooth disease (CMT) 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 P033 CMT1 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Not all exons of *KIF1b* are covered. Some defects in *PMP22* and most defects in *KIF1b* 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. CMT type 2 is an axonal peripheral neuropathy which shows extensive clinical overlap with CMT1. However, in general, the phenotype of CMT2 patients is less severe. Until now, >15 subtypes of CMT2 have been described, each involving a different gene or chromosomal locus. Haploinsufficiency for the *KIF1b* gene is suggested to be responsible for CMT type 2A (Zhao et al. 2001; Drew et al. 2015).

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 CMT1 (IVD)	*PMP22*: all exons *KIF1b*: 2 probes	CMT1A and HNPP 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 CMT1 (IVD)	*PMP22*: all exons *MPZ*: all exons *GJB1*: all exons	CMT1A and HNPP CMT1B 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. MPZ probes in P405-B1 have the same ligation sites as *MPZ* probes in P143-C3.
P143-C3 MFN2-MPZ (RUO)	*MFN2*: all exons *MPZ*: all exons	CMT2A 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 *KIF1b* gene spans ~171 kb on chromosome 1p36.22 and contains 49 exons. The *KIF1b* LRG_252 is identical to GenBank NG_008069.1.

Transcript variants
For *PMP22,* multiple variants have been described (http://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 *KIF1b,* multiple variants have been described (https://www.ncbi.nlm.nih.gov/gene/23095). Transcript variant 1 contains 47 exons and encodes isoform b (NM_015074.3, 10585 nt, coding sequence 253-5565). Transcript variant 3 contains 49 exons, represents the longest transcript and encodes the longest isoform c (NM_001365951.3, 10855 nt, coding sequence 389-5839).

Exon numbering
The *PMP22* exon numbering used in this P033-B4 CMT1 product description is the exon numbering from the LRG_263 sequence. For *KIF1b*, the exon numbering from the RefSeq transcript NM_015074.3 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 P033-B4 CMT1 contains 38 MLPA probes with amplification products between 130 and 436 nucleotides (nt). This includes 16 probes for the common 17p12 deletion/duplication region, two flanking probes for genes outside the common 17p12 deletion/duplication region, and two probes for the *KIF1b* gene. In addition, 18 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 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. 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 P033-B4 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/). No deletions or duplications in *KIF1b* have been reported, however, the association between *KIF1b* mutations and CMT is well established (Zhao et al. 2001, Drew et al. 2015). The analytical sensitivity and specificity for the detection of deletions and duplications in *PMP22* and *KIF1b* 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). In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/ homozygous duplication) may be obtained.

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.

P033 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

- Small (point) mutations in the *PMP22* or *KIF1b* genes will not be detected by using SALSA MLPA Probemix P033 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/KIF1B. 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 P033-B4 CMT1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*KIF1b*	*PMP22* / 17p12
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L00463	5q		
137	Reference probe 03797-L04594	21q		
142	**PMP22 probe** 04656-L04039			**Exon 1**
148	**PMP22 probe** 04657-L04461			**Exon 3**
154	**KIF1b probe** 04681-L04462		**Exon 2**	
160 «	Reference probe 00822-L00130	2p		
166	**PMP22 probe** 04658-L04041			**Exon 3**
172	**PMP22 probe** 11539-L04463			**Exon 4**
178	Reference probe 02958-L02390	7q		
184	**PMP22 probe** 02678-L02158			**Upstream *PMP22***
193	Reference probe 00976-L00563	11p		
202 ∫	**TEKT3 probe** 01460-L00921			17p12, CMT1 region
211	Reference probe 00472-L00088	12q		
220	**KIF1b** probe 04682-L04060		**Exon 46**	
229	**PMP22 probe** 01461-L00926			**Exon 1**
239	**PMP22 probe** 04659-L04464			**Exon 5**
247	Reference probe 00816-L00334	21q		
256	**PMP22 probe** 01462-L00927			**Exon 2**
267	Reference probe 17834-L22900	6q		
274 ¬	DRC3 probe 01452-L00936			17p11, outside CMT1 region
283	Reference probe 08044-L07825	5p		
292	**TEKT3 probe** 04660-L02155			17p12, CMT1 region
302	Reference probe 06487-L06013	1p		
310 ±	**PMP22 probe** 02145-L01641			**Exon 4**
319	Reference probe 01042-L10915	8q		
328	Reference probe 14943-L16676	6q		
337 Δ	**PMP22 probe** 01465-L00930			**Exon 5**
346 ¬	ELAC2 probe 01466-L00917			17p12, outside CMT1 region
355	**PMP22 probe** 02730-L02157			**Upstream *PMP22***
364	Reference probe 15131-L16901	14q		
373	**PMP22 probe** 02729-L02156			**Upstream *PMP22***
381	Reference probe 19749-L26532	9q		
391	**COX10 probe** 01468-L00925			17p12, CMT1 region
400	Reference probe 13588-L15045	1q		
409	Reference probe 00446-L00390	17q		
418	**COX10 probe** 01469-L00924			17p12, CMT1 region
427	Reference probe 17426-L21388	8q		
436	Reference probe 03537-L02903	11p		

[a] See section Exon numbering on page 3 for more information.

± Sequence variant c.260T>C (rs1907114176) could influence the 310 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.
¬ 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 202 nt probe signal may be influenced by the presence of a 9 nt deletion including the ligation site.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 3. P033-B4 probes arranged according to chromosomal location

Table 3a. *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					
418	01469-L00924	*COX10*	NM_001303.4; 1070-1071	CTCCTGGCAGTT-TCCTCATTTCAA	0.2 kb
391	01468-L00925	*COX10*	NM_001303.4; 1273-1274	CCATCAATGCGT-ACATCTCCTACC	1.0 **M**b
		PMP22	**NM_000304.4**		
		stop codon	*688-690 (Exon 5)*		
239	04659-L04464	Exon 5	672-673	ATCTATGTGATC-TTGCGGAAACGC	0.1 kb
337 Δ	01465-L00930	Exon 5	594-595	CTCAACTCGGAT-TACTCCTACGGT	8.5 kb
310 ±	02145-L01641	Exon 4	467-468	CTTCTGCCAACT-CTTCACCCTCAC	0.1 kb
172	11539-L04463	Exon 4	401-400 reverse	TGGTGGCCTGGA-CAGACTGCAGCC	19.5 kb
166	04658-L04041	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)*		
229	01461-L00926	Exon 1	119-120	TTAACATCCCTT-GCATTTGGCTGC	0.1 kb
142	04656-L04039	Exon 1	25-26	ACCACCAGGGAA-CATCTCGGGGAG	2.3 kb
355	02730-L02157	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
202 ∫	01460-L00921	*TEKT3*	NM_031898.3; 1425-1426	TACACGAGGTTG-ACGACACCATCC	27.3 kb
292	04660-L02155	*TEKT3*	NM_031898.3; 267-268	CCCACTCCAATT-TGACCCATAGCC	2.6 **M**b
End of common chromosome 17p12 deletion/duplication region					
274 ¬	01452-L00936	*DRC3*		CGGATCTCCAAG-ATCGACTCCCTG	

Table 3b. *KIF1b* gene

Length (nt)	SALSA MLPA probe	KIF1b exon[a]	Ligation site NM_015074.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	253-255 (Exon 2)		
154	04681-L04462	Exon 2	275-276	CTCAGTGAAGGT-GGCTGTCCGGGT	142.9 kb
220	04682-L04060	Exon 46	5448-5449	CGTGGGGTCCTT-TTGCAGGCCCTC	
		stop codon	5563-5565 (Exon 47)		

[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.

± Sequence variant c.260T>C could influence the 310 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

¬ 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 202 nt probe signal may be influenced by the presence of a 9 nt deletion including the ligation site.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

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.
P405 CMT1	Contains probes for the 17p12 chromosomal region and the *GJB1* and *MPZ* genes.

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P033 product history	
Version	*Modification*
B4	Three reference probes have been replaced.
B3	Three reference probes and the 88 and 96 nt control fragments have been replaced.
B2	Two reference probes at 283 and 301 nt have been replaced. In addition, four extra control fragments have been added at 88-96-100-105 nt.
B1	Probemix has been completely redesigned. Several reference and target probes have been replaced, and more target probes in the CMT/HNPP region have been added.
A1	First release.

Implemented changes in the product description

Version B4-06– 27 January 2023 (04P)
- Table 1 adjusted; probemix versions added and remarks about PMP22 probes corrected.
- Number of exons in the *KIF1b* gene corrected in the Gene structure section.
- Information about transcript variants of *KIF1b* updated in the Transcript variants section.
- Source for exon numbering of the *KIF1b* gene corrected; exon numbering used is from RefSeq transcript NM_015074.3 and not from the LRG_252 sequence.
- Small changes of probe lengths in Table 2 in order to better reflect the true lengths of the amplification products.
- Morocco removed as country with IVD status.
- Various minor textual and layout changes.

Version B4-05– 06 May 2021 (04P)
- Product description rewritten and adapted to a new template.
- Various minor textual or layout changes.
- Warning added to Table 2 and 3a for PMP22 probe (01465-L00930) at 337 nt being more variable.
- Removed P129 as Related SALSA MLPA probemix as this probemix has been discontinued.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version B4-04 – 15 June 2020 (04)
- Colombia and Costa Rica added as countries with IVD status.

Version B4-03 – 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 *PMP22*, *TEKT3* and *COX10* genes updated according to new version of the NM_ reference sequence.

Version B4-02 – 18 December 2018 (02)
- Regulatory status section updated.

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IVD	EUROPE* CE ISRAEL COLOMBIA COSTA RICA
RUO	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.