Product Description
SALSA® MLPA® Probemix P353-B1 CMT4

To be used with the MLPA General Protocol.

Version B1
As compared to version A2, one target probe for *SH3TC2* has been removed and four reference probes have been replaced. For complete product history see page 9.

Catalogue numbers:
- **P353-025R:** SALSA MLPA Probemix P353 CMT4, 25 reactions.
- **P353-050R:** SALSA MLPA Probemix P353 CMT4, 50 reactions.
- **P353-100R:** SALSA MLPA Probemix P353 CMT4, 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 P353 CMT4 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GDAP1*, *MTMR2*, *SBF2*, *SH3TC2*, *EGR2*, and *PRX* genes, which are associated with Charcot-Marie-Tooth neuropathy type 4 (CMT4). In addition, this probemix contains probes for the *NEFL* gene, which is involved in CMT type 1F and 2E.

CMT4 is a group of progressive motor and sensory axonal and demyelinating neuropathies, which is inherited in an autosomal recessive manner. It is characterised by distal muscle weakness and atrophy that is associated with sensory loss and foot deformity. CMT4 is classified into 9 different subtypes (CMT4A to CMT4J). This probemix contains a selection of six genes that are correlated with one of the different subtypes.

The *GDAP1* gene (6 exons) spans ~17 kb of genomic DNA and is located on 8q21.11, 75 Mb from the p-telomere. The *GDAP1* gene encodes a member of the ganglioside-induced differentiation associated protein which is abundantly expressed in brain tissue. CMT4A and intermediated forms of the disorder are caused by mutations in the *GDAP1* gene. This probemix contains one probe for each exon, with the exception of exon 3.

The *MTMR2* gene (15 exons) spans ~91 kb of genomic DNA and is located on 11q21, 95 Mb from the p-telomere. The *MTMR2* gene encodes a protein that belongs to the myotubularin family. CMT4B1 is caused by mutations in the *MTMR2* gene. This probemix contains six probes for this gene.

The *SBF2* gene (40 exons) spans ~515 kb of genomic DNA and is located on 11p15.4, 10 Mb from the p-telomere. The *SBF2* gene encodes a member of the myotubularin-related protein family. Defects in the *SBF2* gene are the cause of CMT4B2. This probemix contains eight probes for this gene.

The *SH3TC2* gene (17 exons) spans ~81 kb of genomic DNA and is located on 5q33.1, 148 Mb from the p-telomere. The gene encodes the SH3TC2 protein which is expressed in Schwann cells of the peripheral nerves. Defects in the *SH3TC2* gene are the cause of CMT4C. This probemix contains seven probes for this gene.

The *EGR2* gene (4 exons) spans ~4 kb of genomic DNA and is located on 10q21.2, 64 Mb from the p-telomere. The gene encodes a member of the early growth response family of proteins, and functions as a transcription factor. Defects in the *EGR2* gene are the cause of CMT4E and CMT1D. This probemix contains one probe for each exon, with the exception of exon 3.

The *PRX* gene (7 exons) spans ~20 kb of genomic DNA and is located on 19q13.2, 46 Mb from the p-telomere. The gene encodes for 2 isoforms, L- and S-periaxin, which are structural proteins required for the maintenance of myelin. CMT4F is caused by mutations in the *PRX* gene. This probemix contains one probe for each exon of this gene.

This probemix furthermore contains probes for all four exons of the *NEFL* gene. The *NEFL* gene spans ~6 kb of genomic DNA and is located on 8p21.2, 25 Mb from the p-telomere. The gene encodes the light chain neurofilament protein. Defects in the *NEFL* gene are the cause of CMT1F and CMT2E.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1468/.

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 *GDAP1*, *MTMR2*, *SBF2*, *SH3TC2*, *PRX*, and *NEFL* exon numbering used in this P353-B1 CMT4 product description is the exon numbering from the LRG_244, LRG_257, LRG_267, LRG_269, LRG_265 and LRG_259 sequences, respectively. Please note that the *EGR2* exon numbering is the exon numbering from the RefSeq transcript NM_001136178.1, not LRG_239. 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 P353-B1 CMT4 contains 49 MLPA probes with amplification products between 131 and 483 nucleotides (nt). This includes 40 probes that target all exons of the *NEFL* and *PRX* genes and several exons of the *GDAP1*, *MTMR2*, *SBF2*, *SH3TC2*, and *EGR2* genes. 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 Charcot-Marie-Tooth neuropathy type 4. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis
Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results
The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure
- In most populations, the major cause of genetic defects in the abovementioned genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P353 CMT4.
- 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.

Inherited Peripheral Neuropathies mutation database:
http://www.molgen.ua.ac.be/CMTMutations/. We strongly encourage users to deposit positive results in the Inherited Peripheral Neuropathies mutation 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 *MTMR2* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P353-B1 CMT4

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	Target
64-105	Control fragments – see table in probemix content section for more information		
131 *	Reference probe 22089-L15549	17q	
136	**GDAP1 probe** 17217-L20544		**Exon 2**
148	**SH3TC2 probe** 11269-L11952		**Exon 9**
154	**PRX probe** 11259-L11942		**Exon 1**
160	Reference probe 12741-L13835	21q	
166	**SBF2 probe** 21596-L30400		**Exon 1**
172	**GDAP1 probe** 11241-L15882		**Exon 5**
178	**SH3TC2 probe** 11272-L11955		**Exon 14**
183	**PRX probe** 11262-L11945		**Exon 4**
190	**SBF2 probe** 11252-L11935		**Exon 35**
195	**MTMR2 probe** 14706-L17527		**Exon 12**
201	**MTMR2 probe** 11253-L17524		**Exon 1**
209	Reference probe 01781-L21728	13q	
215	**NEFL probe** 14108-L20496		**Exon 4**
221 Ж	**PRX probe** 15328-SP0253-L20950		**Exon 2**
226	**EGR2 probe** 14109-L15942		**Exon 4**
232	**SH3TC2 probe** 11268-L17525		**Exon 5**
238	**SBF2 probe** 12814-L20497		**Exon 27**
244	**MTMR2 probe** 11254-L20498		**Exon 2**
250	Reference probe 10808-L20499	4q	
256	**GDAP1 probe** 15329-L17526		**Exon 4**
260	**PRX probe** 21591-L30710		**Exon 3**
267	**NEFL probe** 14110-L15943		**Exon 2**
274	**SH3TC2 probe** 11270-L11953		**Exon 11**
281	**MTMR2 probe** 11255-L11938		**Exon 3**
288 *	Reference probe 17604-L21601	18q	
299	**SBF2 probe** 12812-L19220		**Exon 2**
305	**GDAP1 probe** 11238-L19221		**Exon 1**
312	**EGR2 probe** 14111-L19222		**Exon 2**
320	**SH3TC2 probe** 11271-L11954		**Exon 13**
328	**SBF2 probe** 12813-L15944		**Exon 14**
339	**PRX probe** 11263-L15945		**Exon 5**
346	**EGR2 probe** 17223-L20550		**Exon 1**
355	**GDAP1 probe** 11242-L11925		**Exon 6**
364 *	Reference probe 16279-L18571	20q	
373	**NEFL probe** 14113-L15715		**Exon 3**
382	Reference probe 09147-L03205	7p	
391	**PRX probe** 11265-L15606		**Exon 6c**
400	**SBF2 probe** 14204-L15818		**Exon 22**
409	**SBF2 probe** 17224-L20551		**Exon 11**
419 *	Reference probe 17462-L23159	12p	
427	**MTMR2 probe** 11258-L19224		**Exon 14**
434	**SH3TC2 probe** 11273-L19225		**Exon 16**
443	**SBF2 probe** 21598-L30403		**Exon 17**
448	**MTMR2 probe** 11256-L19227		**Exon 4**
454	**SH3TC2 probe** 15330-L17132		**Exon 2**
463	**PRX probe** 11264-L11947		**Exon 6a**
476	**NEFL probe** 14114-L19228		**Exon 1**
483	Reference probe 16644-L19176	3p	

[a] See section Exon numbering on page 2 for more information.
* New in version B1.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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. P353-B1 probes arranged according to chromosomal location

Table 2a. *SH3TC2*

Length (nt)	SALSA MLPA probe	*SH3TC2* exon[a]	Ligation site NM_024577.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*153-155 (Exon 1)*		
454	15330-L17132	Exon 2	260-261	ATAGCCTCATCT-GAATACAAGGAA	9.4 kb
232	11268-L17525	Exon 5	609-610	TATTGGTGGAGG-ATACAGAGATCC	11.1 kb
148	11269-L11952	Exon 9	1217-1218	CTGGGAAGTGAT-AAGCAGACTGAG	4.4 kb
274	11270-L11953	Exon 11	2672-2673	GTCATCTATAAC-CTCCTGGGACTT	14.5 kb
320	11271-L11954	Exon 13	3235-3236	CACATGCATCAA-GGAGAGCCTGCG	2.4 kb
178	11272-L11955	Exon 14	3399-3400	AGCCTTTGCTGG-CTCTCAAACTTT	3.3 kb
434	11273-L19225	Exon 16	3678-3679	TGGCTACAGTGT-ACTACTCCCTGC	
		stop codon	*4017-4019 (Exon 17)*		

Table 2b. *NEFL*

Length (nt)	SALSA MLPA probe	*NEFL* exon[a]	Ligation site NM_006158.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*355-357 (Exon 1)*		
476	14114-L19228	Exon 1	1188-1189	AACGCTGAGGAA-TGGTTCAAGAGC	1.5 kb
267	14110-L15943	Exon 2	1463-1464	ACGATACCTAAA-AGAATACCAAGA	0.6 kb
373	14113-L15715	Exon 3	1651-1652	CCAGCTCCTATC-TGATGTCCACCC	0.9 kb
215 #	14108-L20496	Exon 4	1970-1971	GGAACAAGCAGC-TAAGAAGAAAGA	
		stop codon	*1984-1986 (Exon 4)*		

Table 2c. *GDAP1*

Length (nt)	SALSA MLPA probe	*GDAP1* exon[a]	Ligation site NM_018972.2	Partial sequence[b] (24 nt a djacent to ligation site)	Distance to next probe
		start codon	*80-82 (Exon 1)*		
305	11238-L19221	Exon 1	84-85	CCCCAAGATGGC-TGAGAGGCAGGA	0.9 kb
136	17217-L20544	Exon 2	271-272	AGTGAGCACAAT-GAGCCTTGGTTT	10.6 kb
256	15329-L17526	Exon 4	616-617	GAAGAAAACCCA-GATTTACAAGAA	1.0 kb
172	11241-L15882	Exon 5	687-688	TGACAATGTCAA-GTATTTGAAGAA	2.1 kb
355	11242-L11925	Exon 6	1864-1865	ATGTCCAGTCAG-TGGGAGGTAGAA	
		stop codon	*1154-1156 (Exon 6)*		

Table 2d. *EGR2*

Length (nt)	SALSA MLPA probe	*EGR2* exon[a]	Ligation site NM_001136178.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*210-212 (Exon 3)*		
346 +	17223-L20550	Exon 1	27-28	TTAGGGGGAGGT-GTGAGTCCTTGG	0.5 kb
312 +	14111-L19222	Exon 2	89-90	TCGGACTCCCTT-CCGAGGCGTCGT	5.5 kb
226 +	14109-L15942	Exon 4	1672-1673	ACCAGCTCCCAA-AGGTCCCGGAGG	
		stop codon	*1638-1640 (Exon 4)*		

Table 2e. *SBF2*

Length (nt)	SALSA MLPA probe	*SBF2* exon[a]	Ligation site NM_030962.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*139-141 (Exon 1)*		
166	21596-L30400	Exon 1	44-45	CCGCTACTGCCG-CTGCAGGGAAAA	100.2 kb
299	12812-L19220	Exon 2	223-224	GGAAAATAATCC-AGAGATTTCCAC	200.9 kb
409	17224-L20551	Exon 11	1262-1263	ATCCTGCCTGCA-ACTTATAAGAAT	24.5 kb
328	12813-L15944	Exon 14	1578-1579	GTTCCACGGCCA-ACAGAAGGATCC	72.5 kb
443	21598-L30403	Exon 17	2045-2046	AGCATTACTCCC-TTTGACCAGTGC	45.9 kb
400	14204-L15818	Exon 22	2926-2927	TCTTCAGAGGAA-CACCCCATGATC	17.7 kb
238	12814-L20497	Exon 27	3695-3696	TGTATGTTGGAA-GAACTCAAGAAG	43.1 kb
190	11252-L11935	Exon 35	4954-4955	AGCACTTCCCCT-CCGAAGACTCTG	
		stop codon	*5686-5688 (Exon 40)*		

Table 2f. *MTMR2*

Length (nt)	SALSA MLPA probe	*MTMR2* exon[a]	Ligation site NM_016156.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*254-256 (Exon 1)*		
201	11253-L17524	Exon 1	285-286	CGAGAGTCTTGG-CTCCCAGCCGGC	35.7 kb
244	11254-L20498	Exon 2	364-365	GAGAATTCAGTG-CATACAAAATCA	22.6 kb
281	11255-L11938	Exon 3	466-467	AACAAGTTAGCA-GAAATGGAAGAA	3.3 kb
448	11256-L19227	Exon 4	562-563	GCTGTACGAGGA-ACTCTGACTGTC	20.7 kb
195	14706-L17527	Exon 12	1675-1676	CATGCAGATGCA-GACAGATCGCCT	5.5 kb
427	11258-L19224	Exon 14	1972-1973	ATGCGCCACCTA-GAGCTCTGGGTG	
		stop codon	*2183-2185 (Exon 15)*		

Table 2g. *PRX*

Length (nt)	SALSA MLPA probe	*PRX* exon[a]	Ligation site NM_181882.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*270-272 (Exon 4)*		
154	11259-L11942	Exon 1	6-7	CCAGGAGCTCTC-GAGGTGTCTGGA	5.0 kb
221 Ж	15328-SP0253-L20950	Exon 2	36-37 and 60-61	TAGAGCGCCGGA-24 nt spanning oligo-GTGACCCTCAGG	0.1 kb
260	21591-L30710	Exon 3	98-99	CGGAAGGGCGTG-AGCCCTGCAGAC	0.2 kb
183	11262-L11945	Exon 4	196-197	GCTCCATGCAGA-ACTGAAGCTGGT	4.2 kb
339	11263-L15945	Exon 5	385-386	GGGCGGCGGCAA-AGAGGGAATCTT	5.0 kb
463	11264-L11947	Exon 6a	499-500	GAACTTCAAGTA-CGAGGACGCACT	1.1 kb
391	11265-L15606	Exon 6c	943-944	TGAGGTGGCTGC-AGGAGCTCGTTT	
		stop codon	*4653-4655 (Exon 6c)*		

[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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
+ These exons are only present in NM_001136178.1, which represents transcript variant 3.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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	Charcot-Marie-Tooth disease type 1A and 2A1. Genes included are: *PMP22, COX10, TEKT3, KIF1b*.
P405 CMT1	Charcot-Marie-Tooth disease type 1A. Genes included are: *GJB1, MPZ, PMP22, TEKT3*.
P143 MFN2-MPZ	Charcot-Marie-Tooth disease type 1B and 2A. Genes included are: *MFN2* and *MPZ*.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P353 CMT4

- Pezzini I et al. (2016). GDAP1 mutations in Italian axonal Charcot–Marie–Tooth patients: Phenotypic features and clinical course. *Neuromuscul Disord*, 26(1), 26-32.

P353 product history	
Version	*Modification*
B1	Compared to previous version, one target probe has been removed and four reference probes have been replaced.
A2	A reference probe has been replaced, and the lengths of several probes have been adjusted.
A1	First release.

Implemented changes in the product description
Version B1-01 – 23 April 2021 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - Warnings removed for salt sensitive probes *Version A2-01 – 09 February 2018 (01P)* - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2). - Salt-sensitivity warning added for the 166 nt probe in Table 1 and 2. - Warning added for the 144 nt probe in Table 1 and 2. *Version 04 – 05 November 2015 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Small changes of the probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Exon numbering of the *GDAP1* and *MTMR2* genes has been changed in Table 1 and 2, according to the NM_ reference sequence. - Ligation sites of the probes targeting the *NEFL* gene updated according to the new version of the NM_ reference sequence. *Version 03 (48)* - Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.

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