Product Description SALSA® MLPA® Probemix P406-A2 CMT2

To be used with the MLPA General Protocol.

Version A2. As compared to version A1, one reference probe has been replaced. For complete product history see page 7.

Catalogue numbers:
- **P406-025R:** SALSA MLPA Probemix P406 CMT2, 25 reactions.
- **P406-050R:** SALSA MLPA Probemix P406 CMT2, 50 reactions.
- **P406-100R:** SALSA MLPA Probemix P406 CMT2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman 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.

General Information: The SALSA MLPA Probemix P406 CMT2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *RAB7A*, *GARS*, *HSPB1*, *HSPB8* and *SPTLC1* genes, which are associated with Charcot-Marie-Tooth hereditary axonal neuropathy type 2.

Charcot-Marie-Tooth hereditary axonal neuropathy type 2 (CMT2) is characterised by distal muscle weakness and atrophy, mild sensory loss and normal or near normal nerve conduction velocities. CMT2 is clinically similar to CMT1, although typically less severe. There are several subtypes of CMT2, which are clinically alike and are only distinguished from each other by molecular genetic findings.

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/

Probemix content: The SALSA MLPA Probemix P406-A2 CMT2 contains 45 MLPA probes with amplification products between 130 and 481 nt. This includes 36 probes for the aforementioned genes. In addition, nine reference probes are included and detect different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

This Probemix contains nine quality control fragments generating amplification products between 64 and 121 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 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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: 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. 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 Biobank (https://catalog.coriell.org) and 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. 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.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 standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes 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 for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous 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 or in or near the *HSPB1* gene. 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 (Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in the genes involved in CMT2 are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemix P406 CMT2.
- 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.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *GARS* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P406-A2 CMT2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)				
		Reference	*RAB7A*	*GARS*	*SPTLC1*	*HSPB1/8*
64-105	Control fragments – see table in probemix content section for more information					
130 *	Reference probe 09978-L10437	19p13				
135	**RAB7A probe** 16316-L21434		**Exon 3**			
142	Reference probe 03797-L23466	21q22				
148 «	**HSPB1 probe** 09540-L19817					*HSPB1* **Exon 3**
156	**GARS probe** 16315-L24244			**Exon 6**		
160	**GARS probe** 16577-L19818			**Exon 1**		
166	**RAB7A probe** 16318-L18707		**Exon 5**			
172	Reference probe 09940-L10399	8q13				
178	**GARS probe** 16320-L18709			**Exon 7**		
184	Reference probe 10973-L11644	14q31				
190	**GARS probe** 16578-L18716			**Exon 13**		
196	**GARS probe** 16322-L18711			**Exon 3**		
202	**SPTLC1 probe** 16323-L18712				**Exon 1**	
208 «	**HSPB1 probe** 09539-L19819					*HSPB1* **Exon 2**
214	**GARS probe** 16324-L18713			**Exon 4**		
220	**GARS probe** 16321-L19068			**Exon 17**		
226	**GARS probe** 16325-L18714			**Exon 9**		
233	**SPTLC1 probe** 16326-L18715				**Exon 8**	
241	**GARS probe** 16329-L19069			**Exon 10**		
247	**SPTLC1 probe** 16330-L19070				**Exon 5**	
256	Reference probe 11349-L12074	12p13				
265	**SPTLC1 probe** 16582-L18717				**Exon 15**	
274	**RAB7A probe** 16332-L18721		**Exon 4**			
283	**SPTLC1 probe** 16334-L19820				**Exon 2**	
292	**GARS probe** 16331-L18720			**Exon 2**		
301	Reference probe 12783-L13918	2q12				
310	**SPTLC1 probe** 16333-L18722				**Exon 11**	
319	**GARS probe** 16580-L19821			**Exon 11**		
328	**HSPB8 probe** 16583-L18726					*HSPB8* **Exon 2**
338	**RAB7A probe** 16335-L19822		**Exon 6**			
346	**GARS probe** 16336-L18725			**Exon 16**		
355	**SPTLC1 probe** 18182-L20492				**Exon 13**	
364	**RAB7A probe** 16340-L18729		**Exon 1**			
373	Reference probe 11001-L11672	4q22				
382	**GARS probe** 16339-L18728			**Exon 14**		
391	**HSPB8 probe** 16584-L18731					*HSPB8* **Exon 1**
400	**RAB7A probe** 16341-L18730		**Exon 2**			
409	**SPTLC1 probe** 16345-L18734				**Exon 6**	
417	**GARS probe** 16343-L18732			**Exon 8**		
427	**GARS probe** 16346-L18735			**Exon 12**		
436	Reference probe 13362-L14792	5p15				
445	**GARS probe** 16579-L15601			**Exon 5**		
454	**GARS probe** 16581-L18733			**Exon 15**		
462 «	**HSPB1 probe** 18273-L23022					*HSPB1* **Exon 1**
481	Reference probe 12565-L13615	13q14				

* New in version A2 (from lot A2-1218 onwards).

« 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.

Table 2. P406-A2 probes arranged according to chromosomal location

Table 2a. *RAB7A*

Length (nt)	SALSA MLPA probe	*RAB7A* exon	Ligation site NM_004637.5	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*233-235 (exon 2)*		
364	16340-L18729	exon 1	146-145 reverse	ACGGAGGACAGA-AGCGAGAAGGTC	69.1 kb
400	16341-L18730	exon 2	265-266	TTGCTGAAGGTT-ATCATCCTGGGA	2.6 kb
135	16316-L21434	exon 3	360-361	CACAATAGGAGC-TGACTTTCTGAC	8.5 kb
274	16332-L18721	exon 4	580-581	CGAGATCCTGAA-AACTTCCCATTT	1.1 kb
166	16318-L18707	exon 5	688-689	ATTCCCTACTTT-GAGACCAGTGCC	5.9 kb
338	16335-L19822	exon 6	919-920	AGGCCTTCAACA-CAATTCCCCTCT	
		stop codon	*854-856 (exon 6)*		

Note: The exon numbering used in this P406-A2 CMT2 product description is the exon numbering from the RefSeq transcript NM_004637.5, which is identical to the LRG_266 sequence. The exon numbering and NM sequence used is from 02/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2b. *GARS*

Length (nt)	SALSA MLPA probe	*GARS* exon	Ligation site NM_002047.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*358-360 (exon 1)*		
160	16577-L19818	exon 1	37-38	TGAAGGTTCTCG-TGTGTCTCGGCC	4.3 kb
292	16331-L18720	exon 2	635-636	AGACGTAGACAA-AGCAGTGGCTGA	1.1 kb
196	16322-L18711	exon 3	707-708	GCCCAAAGATGA-TATTGTAGACCG	1.2 kb
214	16324-L18713	exon 4	865-866	GGCAGCACTTTA-TCCAAGAGGAAC	1.9 kb
445	16579-L15601	exon 5	953-954	AGACAAATTTGC-TGACTTCATGGT	0.4 kb
156	16315-L24244	exon 6	1024-1023 reverse	CATCAATTTCTG-TAAATGAGCTGT	6.2 kb
178	16320-L18709	exon 7	1195-1194 reverse	CTTGAACATTAA-GTTAAAAGACAC	2.5 kb
417	16343-L18732	exon 8	1321-1322	AGTTGCCTTTTG-CTGCTGCCCAGA	3.8 kb
226	16325-L18714	exon 9	1488-1487 reverse	TGGGCTTTTGCT-GAATACAAATAA	1.1 kb
241	16329-L19069	exon 10	1586-1587	AGGCTATTTCAT-TGGCCGCATCTA	4.3 kb
319	16580-L19821	exon 11	1776-1777	TCCTGTCATGCA-CGAGCCACCAAA	0.9 kb
427	16346-L18735	exon 12	1857-1858	TTTGAACCCAGT-AAGGGAGCAATT	3.9 kb
190	16578-L18716	exon 13	11 nt before exon 13	AAATGTGTTTTG-TTTCTTCGTAGG	2.3 kb
382	16339-L18728	exon 14	2067-2066 reverse	ACATTCGGAACA-ACTTCTTCCACT	3.0 kb
454	16581-L18733	exon 15	4 nt after exon 15	GAATTATGTAAG-CAAATTCAATTG	0.8 kb
346	16336-L18725	exon 16	2356-2357	AGATTGGCGTGG-CTTTTGGTGTCA	1.5 kb
220	16321-L19068	exon 17	2536-2535 reverse	CTCTTGCCCTTC-AAACAGAGGATA	
		stop codon	*2575-2577 (exon 17)*		

Note: The exon numbering used in this P406-A2 CMT2 product description is the exon numbering from the RefSeq transcript NM_002047.2, which is identical to the LRG_243 sequence. The exon numbering and NM sequence used is from 02/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2c. *HSPB1*

Length (nt)	SALSA MLPA probe	*HSPB1* exon	Ligation site NM_001540.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*156-158 (exon 1)*		
462 «	18273-L23022	exon 1	53-54	TAGAGACCTCAA-ACACCGCCTGCT	1.3 kb
208 «	09539-L19819	exon 2	577-578	CTTCACGCGGAA-ATACACGTGAGT	0.3 kb
148 « #	09540-L19817	exon 3	755-754 reverse	GCGGCAGTCTCA-TCGGATTTTGCA	
		stop codon	*771-773 (exon 3)*		

« 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.

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.

Note: The exon numbering used in this P406-A2 CMT2 product description is the exon numbering from the RefSeq transcript NM_001540.3, which is identical to the LRG_248 sequence. The exon numbering and NM sequence used is from 02/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2d. *SPTLC1*

Length (nt)	SALSA MLPA probe	*SPTLC1* exon	Ligation site NM_006415.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*39-41 (exon 1)*		
202	16323-L18712	exon 1	1 nt before exon 1	GCACTTTTGGGA-CGCGCTTGTGAC	3.0 kb
283	16334-L19820	exon 2	197-198	TCTGATCTTACA-GTCAAGGTACGA	32.4 kb
247	16330-L19070	exon 5	434-435	GGCGTGGGGACT-TGTGGACCCAGA	12.0 kb
409	16345-L18734	exon 6 (7)	558-557 reverse	AGCAGGAATAGC-ACTGGCTATGGT	12.6 kb
233	16326-L18715	exon 8 (9)	762-763	CTCGGCGTTTCA-TTGTAGTAGAAG	8.2 kb
310	16333-L18722	exon 11 (12)	1077-1076 reverse	AATTGCTGCAGC-AGCTAACAGGGG	8.9 kb
355	18182-L20492	exon 13 (14)	1238-1239	CAACTGGAAGAG-AGCACTGGGTCT	5.8 kb
265	16582-L18717	exon 15 (16)	1431-1432	CGTCCACCATCA-AGGAGGTAGCCC	
		stop codon	*1458-1460 (exon 15)*		

Notes:
- **The *SPTLC1* exon numbering has changed.** From description version 03 onwards (23 September 2016), we have adopted the NCBI exon numbering that is present in the NM_ sequences for this gene. This exon numbering used here may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2d.
- The exon numbering used in this P406-A2 CMT2 product description is the exon numbering from the RefSeq transcript NM_006415.2, which is identical to the LRG_272 sequence. The exon numbering and NM sequence used is from 02/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2e. *HSPB8*

Length (nt)	SALSA MLPA probe	*HSPB8* exon	Ligation site NM_014365.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*524-526 (exon 1)*		
391	16584-L18731	exon 1	419-420	AGCATTTTCGGA-AGCTGAAGAATA	7.8 kb
328	16583-L18726	exon 2	7 nt before exon 2 reverse	TTGCCTGGAAGG-AAGAAAAGTGTG	
		stop codon	*1112-1114 (exon 3)*		

Note: The exon numbering used in this P406-A2 CMT2 product description is the exon numbering from the RefSeq transcript NM_014365.2, which is identical to the LRG_249 sequence. The exon numbering and NM sequence used is from 02/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P033 CMT1: Contains probes to detect genes related to other (sub)types of CMT.
- P405 CMT1: Contains probes to detect genes related to other (sub)types of CMT.
- P143 MFN2-MPZ: Contains probes to detect genes related to other (sub)types of CMT.
- P353 CMT4: Contains probes to detect genes involved in CMT4.
- P129 GJB1: Contains probes for *GJB1*, which is involved in X-linked CMT.

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.

P406 Product history	
Version	*Modification*
A2	*One reference probe has been replaced.*
A1	*First release.*

Implemented changes in the product description

Version A2-01 – 21 February 2019 (01P)
- Product description restructured 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 2c for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 03 – 23 September 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Exon numbering of *SPTLC1* changed.
- Manufacturer's address changed.
- Small textual changes throughout the text.

Version 02 – 30 June 2015 (54)
- Electropherogram picture of the old buffer (introduced Dec. 2012) removed.

More information: www.mlpa.com; www.mlpa.eu	
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