

SALSA MLPA KIT P033-B CMT1/HNPP region

Lot 0406, 0606, 0207.

CHARCOT-MARIE TOOTH DISEASE (CMT) in all of its forms is the most common inherited peripheral neuropathy in humans, with a total prevalence rate of 1 in 2,500. The most common form is CMT1A, in which the average age of onset of clinical symptoms is around 12 years.

CMT1A can be caused by duplication of a 1.5 Mb region on chromosome 17p11.2. A deletion of the same region causes hereditary neuropathy with liability to pressure palsies (HNPP). Duplication or deletion of this chromosomal region is caused by unequal crossing over due to two 24 Kb CMT1A-REP repeats flanking this region.

Increased gene dosage of the PMP22 gene, influencing nerve conduction velocity, is the main cause of the clinical manifestations of CMT1A. PMP22 encodes the peripheral myelin protein 22. Duplication of PMP22 may be found in up to 70% of inherited and 90% of sporadic cases of CMT type 1. Deletion of this region is found in 85% of HNPP cases. The majority of individuals with the HNPP deletion probably remain undiagnosed due to ascertainment bias secondary to the mild phenotype (Lupski, J.R. et al (1993) J. Am. Med. Ass., 270, 2326-2330). Larger regions of duplication or point mutations in PMP22 can also cause CMT1A. Clinically normal adult CMT1A patients are rare, but do exist.

This P033-B CMT probemix contains probes for PMP22, COX, & TEKT3 genes located in the CMT/HNPP region at 17p12. A probe for each exon of the five exons of PMP22 is present in this probemix. In addition this probemix contains several probes just outside the CMT/HNPP region for reference.

This SALSA MLPA kit is designed to detect deletions/duplications of one or more exons of the aforementioned genes. Heterozygote deletions of probe recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. However, mutations and/or polymorphisms very close to the probe ligation site may also result in a reduced relative peak area. Therefore, apparent deletions detected by a single probe always require confirmation by other methods. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Please note that most defects in these genes are expected to be small (point) mutations, most of which will not be detected by this MLPA test.

SALSA® MLPA® kits are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. This kit is not CE/FDA certified for use in diagnostic procedures. SALSA MLPA kits are supplied with all necessary buffers and enzymes. Purchase of the SALSA MLPA test kits includes a limited license to use these products for research purposes.

The use of this MLPA kit requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

Related SALSA MLPA kits

- P143 CMT2A/1B: Charcot-Marie-Tooth disease – genes included: MFN2, MPZ
- P129 GJB1: X-linked Charcot-Marie-Tooth disease – gene included: GJB1
- P064 MR1: Contains 5 probes in the 17p11.2 Smith-Magenis region. The chromosome 17 region deleted in the Smith-Magenis syndrome is immediately adjacent to the CMT1/HNPP chromosomal region.

More information

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References of SALSA MLPA kit P033

- Matejas V. et al. (2006). Identification of Alu elements mediating a partial PMP22 deletion. *Neurogenetics*. 2006 May;7(2):119-26.
- Slater H et al. (2004). Improved testing for CMT1A and HNPP using multiplex ligation-dependent probe amplification (MLPA) with rapid DNA preparations: comparison with the interphase FISH method. *Hum Mutat.*; 24(2):164-71.
- Sutton IJ et al. (2004). Application of multiplex ligation-dependent probe analysis to define a small deletion encompassing PMP22 exons 4 and 5 in hereditary neuropathy with liability to pressure palsies. *Neuromuscul Disord.*; 14(12):804-9.

Data analysis

The P033-B probemix contains 38 different probes with amplification products between 130 and 436 nt. In addition, it contains 5 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt and one synthetic ligation-dependent control fragment at 92 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix can be intra-normalized by dividing the peak area of each probe's amplification product by the total area of only the reference probes in this probemix (block normalization). Secondly, normalisation can be achieved by dividing this intra-normalized probe ratio in a sample by the average intra-normalized probe ratio of all reference samples. Please note that this type of normalization assumes that no changes occurred in the genomic regions targeted by the reference probes.

When only small numbers of samples are tested, visual comparison of peak profiles should be sufficient to easily identify exon deletions. Comparison of results should preferably be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blots or long range PCR.

Note that the Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

This probemix was developed by J.P. Schouten at MRC-Holland. In case the results obtained with this probemix lead to a scientific publication, it would be very much appreciated if the probemix designer could be made a coauthor.

Info / remarks / suggestions for improvement: info@mlpa.com.

SALSA MLPA P033-B CMT1/HNPP region probemix

Length (nt)	SALSA MLPA probe	Chromosomal position	
		Reference	PMP22
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA		
92	Synthetic control fragment		
130	Reference probe 0797-L0463	5q31	
136	Reference probe 3797-L4594	21q22	
142	PMP22 probe 4656-L4039		Exon 1
148	PMP22 probe 4657-L4461		Exon 3
154 ±	KIF1B probe 4681-L4462		1p36
160	Reference probe 0822-L0130	2p21	
166	PMP22 probe 4658-L4041		Exon 3
172	PMP22 probe 4655-L4463		Exon 4
178	Reference probe 2958-L2390	7q31	
184	BX089850 probe 2678-L2158	Close before PMP22 (CMT1 region)	
193	Reference probe 0976-L0563	11p13	
202	TEKT3 probe 1460-L0921	Close before PMP22 (CMT1 region)	
211	Reference probe 0472-L0088	12q14	
219 ±	KIF1B probe 4682-L4060		1p36
229	PMP22 probe 1461-L0926		Exon 1
238	PMP22 probe 4659-L4464		Exon 5
247	Reference probe 0816-L0334	21q11	
256	PMP22 probe 1462-L0927		Exon 2
265	Reference probe 0960-L0547	2p12	
274	Reference probe 1452-L0936	17p11.2 (outside the CMT1 region)	
283 ‡	Reference probe 3010-L2450	9q34	
292	TEKT3 probe 4660-L2155	Close before PMP22 (CMT1 region)	
301	Reference probe 1327-L0872	17p13.3 (outside the CMT1 region)	
310	PMP22 probe 2145-L1641		Exon 4
319	Reference probe 1042-L0791	8q24	
328	Reference probe 0778-L0347	17q21	
337	PMP22 probe 1465-L0930		Exon 5
346	ELAC2 probe 1466-L0917	17p12 (outside the CMT1 region)	
355	FLJ25830 probe 2730-L2157	Close before PMP22 (CMT1 region)	
364	Reference probe 1549-L0992	5q21	
373	FLJ25830 probe 2729-L2156	Close before PMP22 (CMT1 region)	
382	Reference probe 1337-L0880	7q11.23	
391	COX10 probe 1468-L0925	17p12 (CMT1 region)	
400	Reference probe 1088-L0647	17q25.2	
409	Reference probe 0446-L0390	17q21.1	
418	COX10 probe 1469-L0924	17p12 (CMT1 region)	
427	Reference probe 1108-L0679	8q24	
436	Reference probe 3537-L2903	11p15	

‡ Apparently deleted in some samples due to a rare SNP (1:60 in Dutch population).

± Defects in the KIF1B gene have been implicated in Charcot Marie disease type 2A1.

Note: Exon numbering might be different as compared to literature! Please notify us of any mistakes. The identity of the genes detected by the control probes is available on request: info@mlpa.com.

P033-B probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon	Distance to next probe/exon
		<i>P-telomere</i>	3300 Kb
301	1327-L0872	ASPA	9517 Kb
346	1466-L0917	ELAC2 outside common CMT1 deletion-duplication region	1205 Kb
418	1469-L0924	COX10 inside common CMT1 deletion/duplication region	0.2 Kb
391	1468-L0925	COX10 inside common CMT1 deletion/duplication region	1022 Kb
238	4659-L4464	PMP22 exon 5 PMP22 has 5 exons	0.1 Kb
337	1465-L0930	PMP22 exon 5	9 Kb
310	2145-L1641	PMP22 exon 4	0.1 Kb
172	4655-L4463	PMP22 exon 4	19 Kb
166	4658-L4041	PMP22 exon 3	0.1 Kb
148	4657-L4461	PMP22 exon 3	2 Kb
256	1462-L0927	PMP22 exon 2	5 Kb
229	1461-L0926	PMP22 exon 1	0.1 Kb
142	4656-L4039	PMP22 exon 1	2.4 Kb
355	2730-L2157	FLJ25830 (LOC400577)	4.2 Kb
373	2729-L2156	FLJ25830 (LOC400577)	6 Kb
184	2678-L2158	BX089850	24 Kb
202	1460-L0921	TEKT3	27 Kb
292	4660-L2155	TEKT3	2900 Kb
274	1452-L0936	DKFzP586M1120 (just outside the typically deleted region in Smith- Magenis syndrome)	

Based on human genome sequences, PMP22 is located on 17p11.2, approximately 15 Mb from the p-telomere (May 2004).

Note: Exon numbering might be different as compared to literature!
Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA kit P033-B CMT1 sample picture

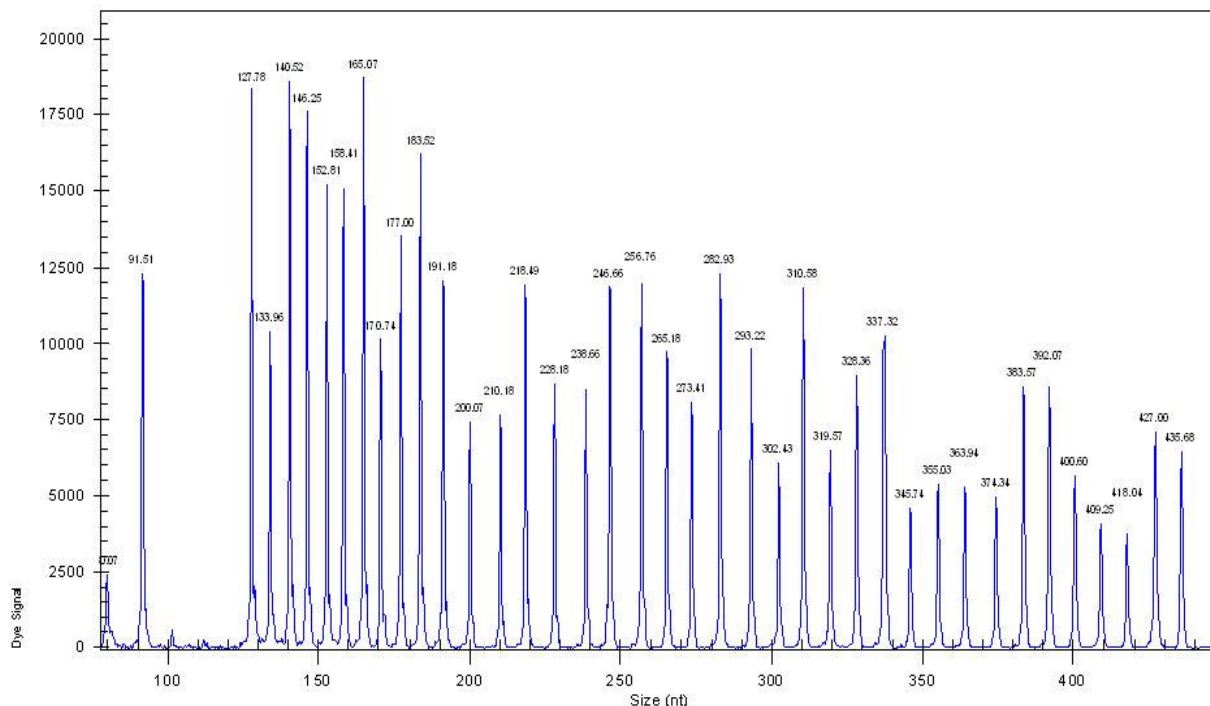


Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analyzed with SALSA MLPA kit P033-B CMT1 (lot 0207).