SALSA MLPA probemix P122-D1 NF1 AREA

Lot D1-1016. As compared to lot C2-0312, four probes in the NF1 area and one reference probe have been removed, four reference probes have been replaced and several probe lengths have been adjusted.

Neurofibromatosis is an autosomal dominant disorder characterised by café-au-lait spots and fibromatous tumours of the skin. Neurofibromatosis type I is caused by defects in the NF1 gene on chromosome 17q11.2. This gene encodes neurofibromin 1, a cytoplasmic protein predominantly expressed in neurons, Schwann cells and leukocytes. Neurofibromatosis type II is caused by defects in the NF2 gene on chromosome 22q12.2.

Deletions of part of the NF1 gene as well as deletions and duplications of the complete NF1 gene have been described. Relatively common (5-10% of NF1 cases) is a deletion of a 1500 kb chromosomal region that includes the NF1 gene. This interstitial 17q11.2 microdeletion arises from unequal crossover between 2 highly homologous 60-kb duplicons. Phenotype of the 17q11.2 microdeletion is usually much more severe than most other NF1 cases and may include severe developmental delay.

The NF1 gene (58 exons) spans ~283 kb of genomic DNA and is located on chromosome 17q11.2. This P122-D1 NF1 area MLPA probemix contains 20 probes for 16 genes located at close distance to NF1 as well as probes for 5 distinct NF1 exons. In addition, 8 references probes are included in this probemix, detecting several different autosomal chromosomal locations. Note that the P081 and P082 probemixes contain probes for each individual NF1 exon.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned region in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak height, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Finally, note that most defects in this gene are expected to be small (point) mutations which will not be detected by this SALSA® MLPA® test.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of a SALSA® MLPA® probemix and reagents 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).

More information

Website : www.mlpa.com
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Related SALSA® MLPA® probemix

- P081/P082 NF1: Neurofibromatosis type 1; these two probemixes contain probes for each NF1 exon.
- P044 NF2: Neurofibromatosis type 2; contains probes for each NF2 exon.

Selected references for SALSA® MLPA® probemix P122 NF1 AREA

- Van Minkelen et al., 2014. A clinical and genetic overview of 18 years neurofibromatosis type 1 molecular diagnostics in the Netherlands. *Clin Genet.* 85:318-327.
- Zickler et al., 2012. Characterization of the nonallelic homologous recombination hotspot PRS3 associated with type-3 NF1 deletions. *Hum Mutat.* 33: 372-383.
- Valero et al., 2011. A highly sensitive genetic protocol to detect NF1 mutations. *J Mol Diagn*. 13:113-122.
- Garcia-Linares et al., 2011. Dissecting Loss of Heterozygosity (LOH) in Neurofibromatosis Type 1-Associated Neurofibromas: Importance of Copy Neutral LOH. *Hum Mutat*. 32:78-80.
- Mautner et al., 2010. Clinical characterization of 29 neurofibromatosis type-1 patients with molecularly ascertained 1.4 Mb type-1 NF1 deletions. *J Med Genet*. 47:623-630.
- Upadhyaya et al., 2009. The spectrum of somatic and germline NF1 mutations in NF1 patients with spinal neurofibromas. *Neurogenetics* 3:251-253.
- Grisart et al., 2008. NF1 microduplication first clinical report: association with mild mental retardation, early onset of baldness and dental enamel hypoplasia? *Eur J Hum Genet*. 16:305-311.
- De Luca et al., 2007. Deletions of NF1 gene and exons detected by multiplex ligation-dependent probe amplification. *J Med Genet*. 44:800-808.

Data analysis

The P122-D1 NF1 area probemix contains 33 MLPA probes with amplification products between 128 and 416 nt. In addition, it contains 9 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA Denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 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 first be normalised intra-sample by dividing the peak height of each probe's amplification product by the total peak height of only the reference probes in this probemix (block normalization). Secondly, inter-sample normalisation can be achieved by dividing this intra-normalized probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes that no changes occurred in the genomic regions targeted by the reference probes.

Data normalisation should 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 blotting, long range PCR, qPCR, FISH.

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

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. For example, a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

This probemix was developed at MRC-Holland.

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

Table 1. SALSA MLPA P122-D1 NF1 AREA probemix

Length (nt)	SALSA MLPA probe	Chromosomal position		
		reference	NF1	NF1 area genes
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA			
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation			
100	X-fragment: Specific for the X chromosome			
105	Y-fragment: Specific for the Y chromosome			
128 *	Reference probe 19616-L26684	4p13		
133 ¥ ±	**TRAF4 probe** 09176-L19109			2348 kb upstream of NF1
142	**NF1 probe** 02491-L01922		**Exon 1**	
147	**NF1 probe** 02512-L01943		**Exon 30**	
154	**NF1 probe** 05220-L03309		**Exon 57**	
160	Reference probe 17436-L21192	16p13		
166	**PSMD11 probe** 09632-L09917			1086 kb downstream of NF1
175	**CRLF3 probe** 03780-L03289			298 kb upstream of NF1
184	**SUZ12 probe** 03786-L03295			628 kb downstream of NF1
190	**SUZ12P probe** 11798-L12590			363 kb upstream of NF1
197	**CPD probe** 09629-L09914			632 kb upstream of NF1
208	**SUZ12P probe** 11801-L12592			336 kb upstream of NF1
220 ±	**RNF135 probe** 03783-L03292			110 kb upstream of NF1
226 ¥	**CPD probe** 09628-L21977			651 kb upstream of NF1
238 *	Reference probe 20555-L14777	1p31		
247	**ATAD5 probe** 03781-L03290			260 kb upstream of NF1
256	**UTP6 probe** 03785-L03294			515 kb downstream of NF1
265	**ASPA probe** 01325-L07456			23001 kb upstream of NF1
274	Reference probe 02470-L01914	15q21		
283	**PMP22 probe** 01463-L00928			11243 kb upstream of NF1
292	**ADAP2 probe** 03782-L03291			168 kb upstream of NF1
301	**LRRC37B probe** 03787-L03296			648 kb downstream of NF1
310	Reference probe 01789-L01353	13q14		
319	**NF1 probe** 02525-L01956		**Exon 49**	
328	**MYO1D probe** 09630-L09915			1420 kb downstream of NF1
337	**NF1 probe** 02507-L01938		**Exon 17**	
346	Reference probe 01232-L00780	10p14		
362	**ZNF207 probe** 09637-L09949			1006 kb downstream of NF1
373	**BLMH probe** 09627-L09912			822 kb upstream of NF1
382 ±	**TRAF4 probe** 08620-L08632			2347 kb upstream of NF1
391	**MYO1D probe** 09631-L09916			1407 kb downstream of NF1
409 *	Reference probe 18498-L23723	19q13		
416 *	Reference probe 20960-L29094	6p12		

* New in version D1 (From lot D1-1016 onwards).
¥ Changed in version D1 (from lot D1-1016 onwards). Small change in length, no change in sequence detected.
± This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. P122 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/ Exon	Genbank #	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
265	01325-L07456	ASPA exon 6	NM_001128085	765-766	GGTCTATAAAAT-TATAGAGAAAGT	11758.7 kb
283	01463-L00928	PMP22 exon 4	NM_000304.2	328-329	GATCTCTGGCAG-AACTGTAGCACC	8995.2 kb
133 ¥ ±	09176-L19109	TRAF4 exon 2	NM_004295.3	20 nt after exon 2	ACACTGCCAGGA-AGAAGCCCAAGC	0.8 kb
382 ±	08620-L08632	TRAF4 exon 4	NM_004295.3	437-438	CAGCTTCAATGT-CATTCCCTGCCC	888.5 kb
373	09627-L09912	BLMH exon 9	NM_000386.3	1330-1331	TGGCTGTGATGT-TGGAAAACACTT	18.9 kb
226 ¥	09628-L21977	CPD exon 12	NM_001304.4	2532-2533	CCAGTGACTACT-TACAAAACTGGA	18.5 kb
196	09629-L09914	CPD exon 21	NM_001304.4	3917-3918	GGTGATAGTCTT-TGACACAGATAA	269 kb
190	11798-L12590	SUZ12P exon 1	NR_024187.2	321 nt before exon 1	GATCTAGACTCT-CTAAACCGCTCG	0.5 kb
208	11801-L12592	SUZ12P exon 3	NR_024187.2	33 nt before exon 3	CCATGGAAATGA-CTTTCTTACTTG	39.2 kb
175	03780-L03289	CRLF3 exon 3	NM_015986.3	471-472	GCTTGGTGGTGT-GGGAGAAGAGAA	37.7 kb
247	03781-L03290	ATAD5 exon 2	NM_024857.4	1334-1335	GCAGGTACGCTT-TAAGACAGTTAC	91.8 kb
292	03782-L03291	ADAP2 exon 3	NM_018404.2	544-545	TGAAGGCCAAGT-TCGAAGCCAGAG	57.8 kb
220 ±	03783-L03292	RNF135 exon 2	NM_032322.3	573-574	GGAACATCTTGT-AGACATTGTCAG	109.9 kb
142	02491-L01922	NF1 exon 1	NM_000267.3	335 nt before exon 1	GCAGAGATCCGC-GCGCTGGGAGAA	130.6 kb
337	02507-L01938	NF1 exon 17	NM_000267.3	2329-2330	GGATCATGAAGA-ATTACTACGTAC	23.8 kb
147	02512-L01943	NF1 exon 30	NM_000267.3	4390-4391	TGAGGAAAACCA-GCGGAACCTCCT	100.1 kb
319	02525-L01956	NF1 exon 49	NM_000267.3	7535-7536	TCACCTGCTATT-GTTGCAAGAACA	11.4 kb
154	05220-L03309	NF1 exon 57	NM_000267.3	8563-8564	TGGAATTGATGA-AGAAACCAGTGA	514.8 kb
256	03785-L03294	UTP6 exon 14	NM_018428.2	1282-1281, reverse	TCCCAGAGTCTC-TAAACAATTCAG	113.1 kb
184	03786-L03295	SUZ12 exon 10	NM_015355.3	1343-1344	CAATGATAAATC-TACGGCTCCTAT	33.2 kb
301	03787-L03296	LRRC37B exon 1	NM_052888.2	426-427	TTCCGCTTCTCA-ACCGGGATCAGA	345.2 kb
362	09637-L09949	ZNF207 exon 9	NM_003457.3	1009-1010	GCTCTGTTTCCT-AGCACAGCACAA	80.2 kb
166	09632-L09917	PSMD11 exon 2	NM_002815.3	180-181	GGAAAACGATGA-AGAGGCAGTGCA	22.1 kb
391	09631-L09916	MYO1D exon 7	NM_015194.2	1064-1065	CTGATGCCATGA-AAGTCATTGGCT	12.9 kb
328	09630-L09915	MYO1D exon 2	NM_015194.2	535-536	CCGCCTCACCTT-TTTGCTATTGCG	

¥ Changed in version D1 (from lot D1-1016 onwards). Small change in length, no change in sequence detected.
± This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

The sequence NM_000267.3 represents transcript variant 2 of NF1 and is a reference standard in the NCBI RefSeqGene project.

Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P122-D1 NF1 sample picture

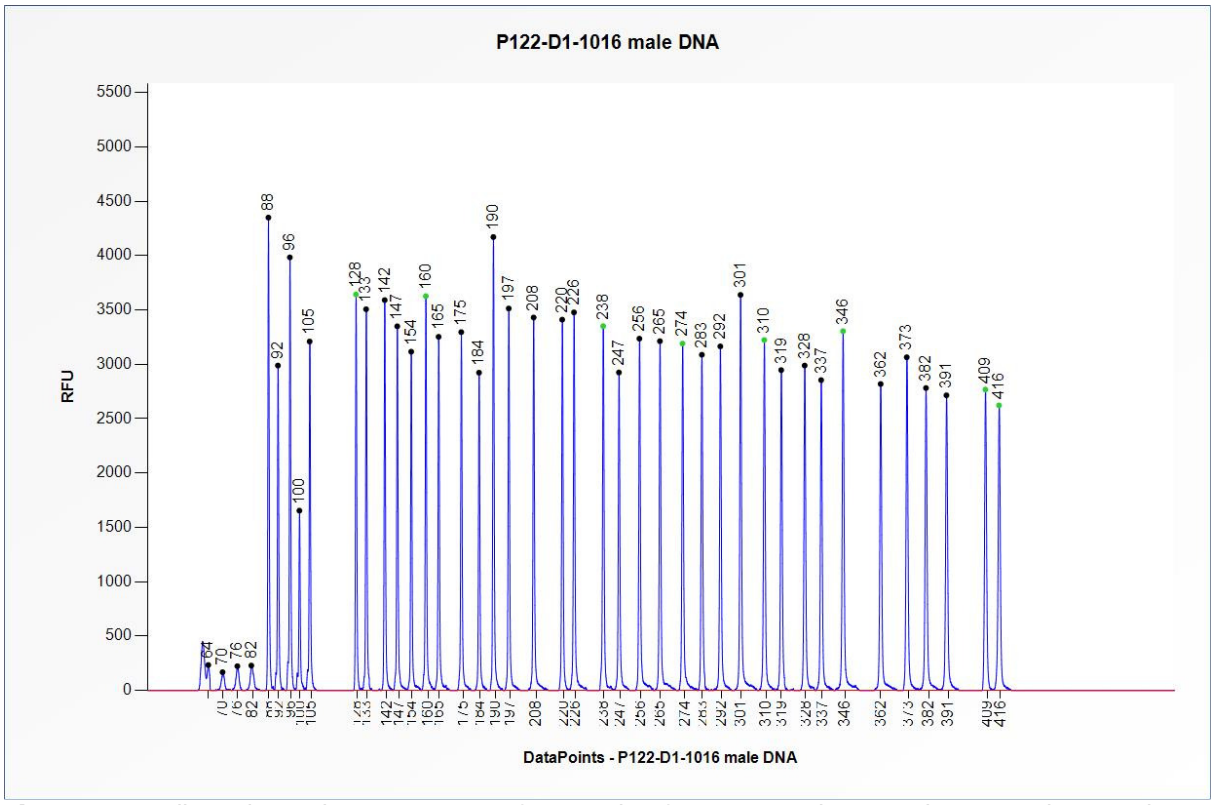

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA P122-D1 (lot D1-1016).

Implemented Changes – compared to the previous product description version.
Version 08 – 18 November 2016 (55) - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included). *Version 07 – 22 July 2015 (54)* - Figure based on the use of old MLPA buffer (replaced in December 2012) removed. - Various minor textual changes throughout the document. *Version 06 (48)* - Warning added in Table 1, 256 nt probe 03785-L03294. *Version 05 (48)* - Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added. *Version 04 (48)* - Various minor textual changes. *Version 03 (46)* - Exon numbering of the CPD and SUZ12P genes has been changed in Table 2. - Various minor textual changes. - Small correction of chromosomal locations in Table 1 and 2. - Remark on RefSeqGene standard and transcript variant added below Table 2. *Version 02 (46)* - New reference added on page 1. - Partial sequence for probe 02491-L01922 corrected. - Exon numbering has been changed on page 3 and 4. - Gene names updated. - Ligation sites updated according to new version of the NM_reference sequence. - Small changes of probe lengths in Table 2 in order to better reflect the true lengths of the amplification products. - Various minor textual changes on page 1 and 2. - Various minor layout changes.