SALSA MLPA probemix P044-B3 NF2

Lot B3-1016: As compared to the previous version (lot B2-1112), two reference probes have been replaced and several probe lengths have been adjusted.

Neurofibromatosis type 2 (NF2) is an autosomal dominant cancer syndrome that predisposes the development of bilateral vestibular schwannomas. This disease is caused by inactivating mutations of the NF2 tumour-suppressor gene. Mutational analysis of the NF2 gene in typical NF2 patients has demonstrated causative mutations in as many as two-thirds of individuals (Jacoby, L.B. et al., 1997. *Am J Hum Genet*.). Many studies have documented that the NF2 gene behaves as a typical tumour-suppressor gene in these patients, with first hits detectable in both constitutional and tumour specimens and second hits detectable only in tumours. Kluwe, L. et al. (2005, *Genes Chromosomes Cancer*) showed that large alterations affecting the NF2 gene account for 27% of the 77 cases in which no intragenic small mutations were found, and for 16% of the total of 134 mutations identified among the 188 NF2 patients screened in the study.

The NF2 gene (17 exons) spans ~95 kb of genomic DNA and is located on 22q12.2, ~30 Mb from the p-telomere. The P044-B3 NF2 probemix contains probes for each of the exons of the NF2 gene. In addition, two probes located in genes upstream of the NF2 gene are included, and one downstream. Eleven reference probes are included detecting different autosomal chromosomal regions.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned gene 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).

Related SALSA® MLPA® probemixes

- P081/P082 NF1: Neurofibromatosis type I – gene included: NF1.
- P122 NF1 AREA: Neurofibromatosis type I – NF1 region.
- P455 LZTR1: Schwannomatosis – gene included: LZTR1.

More information

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References for SALSA® MLPA® probemix P044 NF2

- Luis, L. et al., 2013. NF2 Genetic Alterations in Sporadic Vestibular Schwannomas: Clinical Implications. *Otology & Neurotology*. 34:1355-61.
- Abo-Dalo, B. et al., 2010. Large intragenic deletions of the NF2 gene: breakpoints and associated phenotypes. *Genes Chromosomes Cancer* 49:171-5.
- Evans, D. G. et al., 2007. Mosaicism in NF2: an update of risk based on uni/bilaterality of vestibular schwannoma at presentation and sensitive mutation analysis including MLPA. *J Med Genet* 44:424-8.
- Kluwe, L. et al., 2005. Screening for large mutations of the NF2 gene. *Genes Chromosomes Cancer* 42:384-91.

Data analysis

The P044-B3 NF2 probemix contains 33 MLPA probes with amplification products between 130 and 418 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 intra-sample normalised 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 normalisation). Secondly, inter-sample normalisation can be achieved by dividing this intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes no changes occurred in the genomic regions recognised 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 results 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 disease. 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 P044-B3 NF2 probemix

Length (nt)	SALSA MLPA probe	Chromosomal position reference	NF2
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		
130	Reference probe 00797-L00463	5q31	
139	**NF2 probe** 01563-L01134		**Upstream**
148	**NF2 probe** 01564-L04978		**Exon 1**
157	Reference probe 13447-L19445	17p13	
166	**NF2 probe** 01565-L01137		**Exon 2**
175	**NF2 probe** 01566-L01138		**Exon 3**
184 *	Reference probe 10973-L11644	14q31	
193	**NF2 probe** 01567-L01139		**Exon 4**
202	**NF2 probe** 01568-L01140		**Exon 5**
211	Reference probe 01484-L01092	16q24	
220	**NF2 probe** 01569-L02031		**Exon 6**
226 ¥	**NF2 probe** 18696-L29634		**Exon 7**
238 *	Reference probe 14970-L16706	6q22	
247	**NF2 probe** 01571-L01143		**Exon 8**
256	**NF2 probe** 01572-L01144		**Exon 9**
265	Reference probe 03075-L02475	5p15	
274	**NF2 probe** 02485-L01984		**Exon 10**
280	**NF2 probe** 15774-L17826		**Exon 11**
292 ¬	**AP1B1 probe** 15529-L17384		**Centromeric**
300	**NF2 probe** 01575-L01147		**Exon 12**
309	**NF2 probe** 01576-L01148		**Exon 13**
319	Reference probe 01042-L10915	8q24	
328	**NF2 probe** 01577-L01149		**Exon 14**
337	**NF2 probe** 01578-L01150		**Exon 15**
346	Reference probe 03580-L02941	3p22	
355	**NF2 probe** 03318-L02736		**Exon 16c**
366 ¥	**NF2 probe** 01580-L29633		**Exon 17**
375 ¥	Reference probe 00655-L18157	4q27	
382 ±	**NF2 probe** 01581-L01135		**Upstream**
391 ¬ «	**NIPSNAP1 probe** 02580-L02042		**Centromeric**
400	Reference probe 14839-L16547	1p34	
408 ¬ «	**CABP7 probe** 03317-L02735		**Telomeric**
418	Reference probe 03920-L03375	15q21	

* New in version B3 (from lot B3-1016 onwards).
¥ Changed in version B3 (from lot B3-1016 onwards). Change in length, no change in sequence detected.
¬ Flanking probe. Included to facilitate determination of the extend of a deletion / duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition being tested.
± SNP rs1800538 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.

Note: Exon numbering used here may differ from literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. NF2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	NF2 Exon	Ligation site NM_000268.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
292 ¬	15529-L17384	AP1B1 gene		CTTGGCACCAAA-ATGTCCGCGGCC	167.3 kb
391 ¬ «	02580-L02042	NIPSNAP1 gene		AGGCTGACAAGT-TCTGAGGATTAC	47.4 kb
		Start codon	*444-446 (exon 1)*		
139	01563-L01134	Upstream	411 nt before exon 1	ACGCAGTCCCCT-GGGGCGCCACAC	0.1 kb
382 ±	01581-L01135	Upstream	314 nt before exon 1	CCTCTAAGTGGT-TTCCCGGGTAAG	0.8 kb
148	01564-L04978	Exon 1	487-488	CAGCTCTCTCAA-GAGGAAGCAACC	32.8 kb
166	01565-L01137	Exon 2	628-629	AACCTGGTTCTT-TGGACTGCAGTA	2.3 kb
175	01566-L01138	Exon 3	759-760	CTGAGAATGCTG-AAGAGGAGCTGG	3.1 kb
193	01567-L01139	Exon 4	854-855	CCTCCTGAGGCT-TCTGTGCTCCTG	12.4 kb
202	01568-L01140	Exon 5	915-916	ACCCCAGTGTTC-ACAAGCGGGGAT	1.0 kb
220	01569-L02031	Exon 6	1002-1003	TGTGGGAGGAGA-GAATTACTGCTT	2.6 kb
226 ¥	18696-L29634	Exon 7	1070-1071	GAATATCTGAAG-ATAGCTCAGGAC	3.0 kb
247	01571-L01143	Exon 8	1171-1172	GGGGCTTCACAT-TTATGACCCTGA	3.7 kb
256	01572-L01144	Exon 9	68 nt before exon 9	TTGCGCATTTGT-GGAATTTCCAAT	3.5 kb
274	02485-L01984	Exon 10	1434-1433, reverse	CACCTGCTTTCT-AGCCTTCTCCTC	3.5 kb
280	15774-L17826	Exon 11	1546-1545, reverse	CGTTGGCCATTG-TTGCTTCTTCTT	1.4 kb
300	01575-L01147	Exon 12	1624-1625	CACCGAGGAGGA-GGCAAAACTTCT	1.5 kb
309	01576-L01148	Exon 13	1813-1814	GCTGAAGCAGGA-CCTGCAGGAAGC	3.4 kb
328	01577-L01149	Exon 14	1945-1946	CTTCAACCTCAT-TGGTGACAGCCT	3.2 kb
337	01578-L01150	Exon 15	2071-2072	CAATGAACTCAA-GACAGAAATCGA	2.0 kb
355 #	03318-L02736	Exon 16c	1.9 kb after exon 15; NM_181825.2; 2622-2623	CTGGTGTGTTTA-ACTCAAGATCAA	11.4 kb
366 ¥	01580-L29633	Exon 17	2304-2305	CAGATATCAAGA-GAGCCATCCATA	34.3 kb
		Stop codon	*2229-2231 (exon 17)*		
408 ¬ «	03317-L02735	CABP7 gene		ACATAGAGAACA-TCATCATGACGG	

¥ Changed in version B3 (from lot B3-1016 onwards). Change in length, no change in sequence detected.
¬ Flanking probe. Included to facilitate determination of the extend of a deletion / duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition being tested.
« 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.
± SNP rs1800538 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
Exon 16 is not present in transcript variant 1 (NM_000268.3). This probe is located in exon 16c of the NM_181825.2 transcript.

The NM_000268.3 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Note: Exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P044-B3 NF2 sample picture

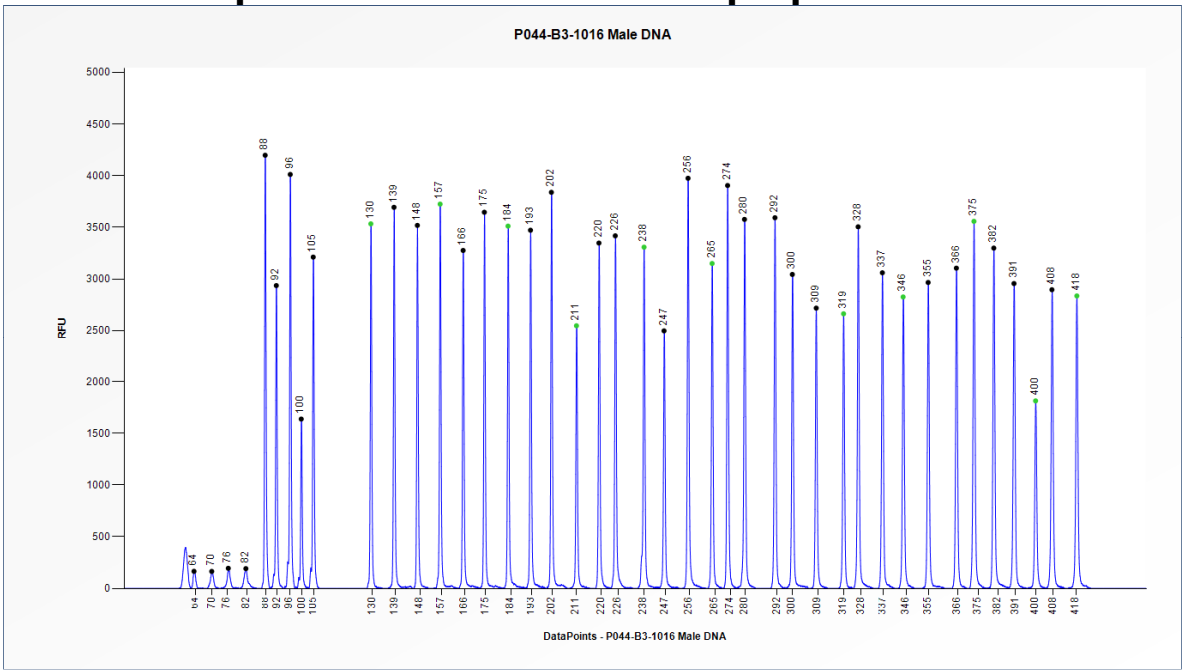

Figure 1. Capillary electrophoresis pattern of a sample of approximately 50 ng human male control DNA analysed with SALSA MLPA probemix P044-B3 NF2 (lot B3-1016).

Implemented Changes – compared to the previous product description versions.

Version 16 – 24 November 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Notation changed of promoter and flanking probes into upstream and centromeric/telomeric

Version 15 – 12 August 2015 (54)
- Various minor textual changes.
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.
- "Peak area" replaced with "peak height".

Version 14 (49)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 13 (48)
- Warning added in Table 1, 391 nt probe 02580-L02042 and 408 nt probe 03317-L02735.

Version 12 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 11 (48)
- Various minor textual changes.
- Ligation sites updated according to new version of the NM_reference sequence.
- Remark on RefSeqGene standard and transcript variant added below Table 2.

Version 10 (46)
- Various minor textual changes.
- Mistake corrected on page 2 (NF1 changed to NF2).

Version 09 (46)
- Warning added for a SNP below table 1 and 2 for probe 01581-L01135 at 382 nt.

Version 08 (46)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the

amplification products.
- Ligation sites updated according to new version of the NM_reference sequence.