Product Description SALSA® MLPA® Probemix P081-D1 NF1 mix 1 & P082-C2 NF1 mix 2

To be used with the MLPA General Protocol.

P081 version D1. As compared to version C1, exon 21 and exon 23 probes have been replaced, a probe for exon 21 has been added, a reference probe has been replaced and several probes have a small change in length. For complete product history see page 10.

P082 version C2. As compared to version C1, two reference probes have been replaced and one probe has a small change in length. For complete product history see page 10.

Catalogue numbers:

- **P081-025R:** SALSA MLPA probemix P081 NF1 mix 1, 25 reactions.
- **P081-050R:** SALSA MLPA probemix P081 NF1 mix 1, 50 reactions.
- **P081-100R:** SALSA MLPA probemix P081 NF1 mix 1, 100 reactions.

- **P082-025R:** SALSA MLPA probemix P082 NF1 mix 2, 25 reactions.
- **P082-050R:** SALSA MLPA probemix P082 NF1 mix 2, 50 reactions.
- **P082-100R:** SALSA MLPA probemix P082 NF1 mix 2, 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.

Intended use: The SALSA MLPA probemix P081 NF1 mix 1 and P082 NF1 mix 2 are an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *NF1* gene in order to confirm a clinical diagnosis of Neurofibromatosis type 1. This assay is for use with human DNA derived from peripheral blood and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials.
Deletions or duplications detected with the P081 NF1 mix 1 and P082 NF1 mix 2 probemixes should be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in the *NF1* gene are point mutations, which will not be detected by MLPA. It is therefore recommended to use these SALSA MLPA probemixes in combination with sequence analysis of the *NF1* gene. These probemixes are not intended to be used as standalone assays for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Neurofibromatosis is an autosomal dominant disorder characterised particularly by café-au-lait spots and fibromatous tumours of the skin. Neurofibromatosis type 1 is caused by loss-of-function mutations in the *NF1* gene on 17q11.2. Neurofibromatosis type 2 is caused by defects in the *NF2* gene on chromosome 22q12.2, for which the P044 NF2 MLPA probemix (RUO) can be used.
Estimated birth incidence of Neurofibromatosis type 1 is 1 in 3000, with about half of the NF1 cases caused by *de novo* sporadic mutations. *De novo* sporadic mutations may also be the result of germline mosaicism in apparently unaffected parents.

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 1.4 Mb chromosomal region harbouring multiple genes, including the *NF1* gene. The phenotype of this 17q11.2 microdeletion is usually much more severe than most other NF1 cases and may include developmental delay. Next to the 1.4 Mb deletion described above, a 1.2 Mb microdeletion and nonrecurrent atypical microdeletions of different sizes have been reported. The P122 NF1 area MLPA probemix (RUO) can be used to determine the extent of the deletion as it contains many probes for other genes in the frequently deleted 1.4 Mb region.
More information is available on https://www.ncbi.nlm.nih.gov/books/NBK1109/.

Gene structure: The *NF1* gene spans ~300 kb on chromosome 17q11.2. The *NF1* LRG_214 sequence is available at http://www.lrg-sequence.org/ and is identical to the NCBI NG_009018.1 sequence.

Transcript variants: https://www.ncbi.nlm.nih.gov/gene/4763 describes several transcript variants of the *NF1* gene. The NM_000267.3 sequence represents transcript variant 2. This sequence is a reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 1 (384-386) and the stop codon is located in exon 58 (8838-8840). The NM_001042492.2 sequence represents transcript variant 1. This transcript variant contains an additional in-frame coding exon (31).

Exon numbering: The exon numbering used in this P081-D1/P082-C2 NF1 product description is the exon numbering from the NCBI NG_009018.1 which is identical to the LRG_214 sequence. The exon numbering and NM sequence used is from 04/2018, but can be changed (e.g. by NCBI) after the release of the product description.

P081-D1 and P082-C2 probemix content: The P081 and P082 probemixes together contain one probe for each exon, three probes for exon 1, one probe for intron 1, and two probes for the exons 15, 21, 23, 51 and 58 of the *NF1* gene. Additionally, these probemixes contain one upstream and one downstream probe and two probes for the *OMG* gene, located within intron 36 of the *NF1* gene.

The P081-D1 probemix contains 46 MLPA probes with amplification products between 130 and 463 nt in length, including 11 reference probes. The P082-C2 probemix contains 44 MLPA probes with amplification products between 130 and 483 nt in length, nine of which are reference probes. 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 105 nt: four DNA Quantity Fragments (Q-fragments), two DNA Denaturation Fragments (D-fragments), one benchmark fragment, 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 and 96 fragment 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.mlpa.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 from peripheral blood, 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 Neurofibromatosis type 1. 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.

Performance characteristics: The expected number of *NF1* deletions/duplications which can be detected with these MLPA probemixes is approximately 10% of all *NF1* mutations in most patient populations. Analytical performance for the detection of deletions/duplications in the *NF1* gene is very high and can be considered >99% (based on a 2006-2015 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must 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 expected results for the *NF1* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication).

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 *NF1* 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 in some cases 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 *NF1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P081/P082 NF1.
- 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: Mosaic *NF1* deletions obtained with the P081/P082 NF1 probemixes must be confirmed by analysis of a second, independently collected DNA sample or a different technique, in order to exclude a false positive mosaic result.

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 one or 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.

NF1 mutation database: http://www.LOVD.nl/NF1. We strongly encourage users to deposit positive results in the LOVD 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 SNPs and unusual results (e.g., a duplication of *NF1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P081-D1 NF1 mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a] Reference	NF1
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136 « ¬	**NF1 probe** 18363-L23328		**Downstream**
142 ¥	**NF1 probe** 02491-L29974		**Exon 1**
148	**NF1 probe** 18364-L23329		**Exon 28**
154	**NF1 probe** 05220-L03309		**Exon 57**
160	**NF1 probe** 02493-L01924		**Exon 2**
166	**NF1 probe** 02513-L01944		**Exon 32**
172	Reference probe 09940-L29795	8q13	
178	**NF1 probe** 02865-L02617		**Exon 4**
184	**NF1 probe** 18367-L23332		**Exon 35**
190	Reference probe 09836-L10246	11q24	
196 ¬	**NF1 probe** 18368-L23333		**Upstream**
202	**NF1 probe** 02497-L03706		**Exon 6**
208 «	**NF1 probe** 19361-L26126		**Exon 58**
214	**NF1 probe** 02517-L26127		**Exon 37**
220	**NF1 probe** 18032-L22398		**Exon 7**
226	**NF1 probe** 19363-L25737		**Exon 51**
232	**NF1 probe** 13221-L26128		**Exon 11**
238	**NF1 probe** 02519-L01950		**Exon 39**
244 *	**NF1 probe** 21185-L29794		**Exon 21**
250 ¥	**NF1 probe** 03849-L18072		**Exon 26**
256 ¥ Ж	**NF1 probe** 18033-SP0601-L29798		**Exon 24**
264	Reference probe 09265-L10877	10q21	
272	**NF1 probe** 02521-L22646		**Exon 41**
279	Reference probe 12437-L13438	14q24	
289	**NF1 probe** 04071-L01954		**Exon 47**
298	**NF1 probe** 02503-L22647		**Exon 13**
304	Reference probe 16436-L18889	18q21	
312	**NF1 probe** 04076-L22649		**Exon 15**
319	**NF1 probe** 02525-L22650		**Exon 49**
328	Reference probe 05388-L04785	12p11	
337 *	**NF1 probe** 21000-L29222		**Exon 23**
346	**NF1 probe** 02526-L01957		**Exon 50**
353	**NF1 probe** 02507-L22658		**Exon 17**
364 *	Reference probe 05953-L05397	2p22	
373	**NF1 probe** 02528-L01959		**Exon 52**
382 *	**NF1 probe** 21186-L29799		**Exon 21**
391 «	**NF1 probe** 02530-L01961		**Exon 58**
400	**NF1 probe** 04072-L03709		**Exon 29**
409	Reference probe 08725-L08736	9q21	
418 Ж	**NF1 probe** 18408-SP0653-L23405		**Exon 23**
427	**NF1 probe** 12024-L26426		**Exon 18**
436	**NF1 probe** 03853-L29796		**Exon 42**
445	Reference probe 05026-L29797	2q32	
454 Ø	**OMG probe** 04075-L03310		**Intron 36 of NF1**
463	Reference probe 09908-L10321	16p13	

(a) The exon numbering used in this P081-D1/P082-C2 NF1 product description is the exon numbering from the NCBI NG_009018.1 which is identical to the LRG_214 sequence. The exon numbering and NM sequence used is from 04/2018, but can be changed (e.g. by NCBI) after the release of the product description.

* New in version D1 (from lot D1-0617 onwards).
¥ Changed in version D1 (from lot D1-0617 onwards). Small change in length, no change in sequence detected.
« 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.

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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Probe detects the *OMG* gene, located within intron 36 of *NF1* gene. We have no information on the clinical significance of copy number changes of only the *OMG* gene.

Table 1b. SALSA MLPA Probemix P082-C2 NF1 mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a]	
		Reference	NF1
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
138 ¥	**NF1 probe** 18382-L19008		**Exon 1**
147	**NF1 probe** 02512-L01943		**Exon 30**
154	**NF1 probe** 12018-L12866		**Exon 53**
160	**NF1 probe** 02494-L01925		**Exon 3**
166	**NF1 probe** 02514-L01945		**Exon 34**
172	**NF1 probe** 18173-L22738		**Exon 5**
178	Reference probe 11571-L12318	16q21	
184	Reference probe 17862-L22121	19q13	
190	**NF1 probe** 12019-L12867		**Intron 1**
197	**NF1 probe** 18374-L26502		**Exon 36**
205	**NF1 probe** 02498-L22716		**Exon 8**
211	**NF1 probe** 02518-L01949		**Exon 38**
220	Reference probe 12427-L13428	22q12	
227	**NF1 probe** 19362-L26201		**Exon 1**
233	**NF1 probe** 02500-L26202		**Exon 10**
241	**NF1 probe** 02520-L26200		**Exon 40**
249	**NF1 probe** 12021-L26199		**Exon 44**
257	**NF1 probe** 03778-L26198		**Exon 12**
265	**NF1 probe** 02522-L01953		**Exon 46**
271	Reference probe 15957-L26197	6q15	
281 Ж	**NF1 probe** 19364-SP0809-L25738		**Exon 15**
292	**NF1 probe** 02504-L26817		**Exon 14**
300	**NF1 probe** 02524-L22720		**Exon 48**
307	**NF1 probe** 18034-L22721		**Exon 54**
317 Ж	**NF1 probe** 18369-SP0646-L23334		**Exon 16**
328	**NF1 probe** 13217-L22725		**Exon 51**
337	**NF1 probe** 18370-L23335		**Exon 20**
345	**NF1 probe** 02529-L01960		**Exon 55**
353	Reference probe 06708-L26176	10p11	
362 Ж	**NF1 probe** 18174-SP0619-L22739		**Exon 25**
372	Reference probe 08893-L23475	14q24	
382	**NF1 probe** 18035-L22401		**Exon 56**
391	**NF1 probe** 18365-L23330		**Exon 33**
400 *	Reference probe 07808-L23525	3p22	
409	**NF1 probe** 18170-L26175		**Exon 27**
419	**NF1 probe** 03854-L23156		**Exon 43**
427	**NF1 probe** 12025-L23157		**Exon 19**
436	**NF1 probe** 18036-L22765		**Exon 22**
444 Ø	**OMG probe** 04069-L03311		**Intron 36 of NF1 (OMG gene)**
454	**NF1 probe** 03856-L03307		**Exon 45**
463 Ж	NF1 probe 18037-SP0602-L22403		**Exon 9**
472	**NF1 probe** 18038-L26174		**Exon 31**
483 *	Reference probe 06676-L06254	11p15	

(a) The exon numbering used in this P081-D1/P082-C2 NF1 product description is the exon numbering from the NCBI NG_009018.1 which is identical to the LRG_214 sequence. The exon numbering and NM sequence used is from 04/2018, but can be changed (e.g. by NCBI) after the release of the product description.

* New in version C2 (from lot C2-0317 onwards).
¥ Changed in version C2 (from lot C2-0317 onwards). Small change in length, no change in sequence detected.
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.
Ø Probe detects the *OMG* gene, located within intron 36 of *NF1* gene. We have no information on the clinical significance of copy number changes of only the *OMG* gene.

Table 2. NF1 probes arranged according to chromosomal location

Length (nt) P081	Length (nt) P082	SALSA MLPA probe	Exon[a]	Ligation site[b] NM_000267.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
			start codon	*384-386 (ex 1)*		
196 ¬		18368-L23333	Upstream	8.0 kb before exon 1	CAAAGCAAGTTC-AGCATCAGAGGA	7.7 kb
142		02491-L29974	Exon 1	335 nt before exon 1	GCAGAGATCCGC-GCGCTGGGAGAA	0.4 kb
	227	19362-L26201	Exon 1	53-54	AAGGATCCCACT-TCCGGTGGGGTG	0.3 kb
	138	18382-L19008	Exon 1	415-414 reverse	TGACCACGGCCT-GGACCCATTCCA	0.6 kb
	190	12019-L12867	Intron 1	597 nt after exon 1	TCGTCTCATCCT-GCCCCGAGAGCT	60.1 kb
160		02493-L01924	Exon 2	475-476	GCAGAACACACA-TACCAAAGTCAG	3.0 kb
	160	02494-L01925	Exon 3	631-632	ATATCTCTCTCA-GTTGATTATATT	4.2 kb
178		02865-L02617	Exon 4	735-736	TGCCAGAAATCT-GCCATTTTCTTC	6.7 kb
	172	18173-L22738	Exon 5	958-959	AAAATTAAAACG-ACTCCTGAAGGG	11.5 kb
202		02497-L03706	Exon 6	1000-1001	AGCCCTAAAGAA-GGTTGCGCAGTT	0.3 kb
220		18032-L22398	Exon 7	1046-1047	TAGGCATTTTGG-AACTGGGTAGAA	0.9 kb
	205	02498-L22716	Exon 8	1167-1168	AAAGCACCAAAC-GTAAAGCAGCAG	17.9 kb
	463 Ж #	18037-SP0602-L22403	Exon 9	1341-1342; 1372-1373	TGACAGAAAGTG-31 nt spanning oligo-AAGTACTTACAT	0.6 kb
	233 #	02500-L26202	Exon 10	1508-1509	GATGTGGATCTA-ATGATTGACTGC	0.7 kb
232 #		13221-L26128	Exon 11	291 nt after exon 11	TGAGAAAAATGT-CACTGAAAATAC	4.5 kb
	257	03778-L26198	Exon 12	1722-1723	TTGGTGAAACAC-TTCATAAAGCAG	8.2 kb
298 #		02503-L22647	Exon 13	1849-1850	GACAAGAAGCTA-TAAGTATCTTCT	4.6 kb
	292	02504-L26817	Exon 14	1985-1986	CAACTGGTCCCT-CAGTCACACATG	2.5 kb
312		04076-L22649	Exon 15	235 nt before exon 15 reverse	TAACTGGCATGT-ACATATAAAGCT	0.3 kb
	281 Ж #	19364-SP0809-L25738	Exon 15	2053-2054; 2083-2084	TCAGTTAGATAG-30 nt spanning oligo-AGAAACATTTTG	1.5 kb
	317 Ж #	18369-SP0646-L23334	Exon 16	35 nt before exon 16; 2107-2108	TTAGGTTATTGA-38 nt spanning oligo-ACAAATGCTTTT	1.8 kb
353		02507-L22658	Exon 17	2329-2330	GGATCATGAAGA-ATTACTACGTAC	1.4 kb
427 #		12024-L26426	Exon 18	2587-2588	CTTGCCCAACTA-TAACACATTCAT	0.7 kb
	427 #	12025-L23157	Exon 19	2 nt after exon 19	AACACTGAGGTA-TGCCCTTAGCAA	0.2 kb
	337	18370-L23335	Exon 20	34 nt before exon 20	AGCTCTAGACTA-AGTTGCTTTCAA	1.8 kb
244 #		21185-L29794	Exon 21	3064-3063 reverse	GCCGATCCATAA-ATTTGCTGACAG	0.1 kb
382 #		21186-L29799	Exon 21	3170-3171	AGTCCTGCTCTG-TATCCAATGCTA	0.4 kb
	436 #	18036-L22765	Exon 22	3269-3270	CAATTTGTAGAA-CAAACCATAGCT	0.5 kb
418 Ж #		18408-SP0653-L23405	Exon 23	3434-3435; 3468-3469	AAACTGTGTCAA-34 nt spanning oligo-TCTCATTTTGCC	0.2 kb
337		21000-L29222	Exon 23	179 nt after exon 23	CCTGTGACAATG-CTCCCTTTTTCT	0.2 kb
256 Ж #		18033-SP0601-L29798	Exon 24	37 nt before exon 24; 3498-3499	GGCTTCAAAAAC-39 nt spanning oligo-ATAAGATGGTAG	1.4 kb
	362 Ж	18174-SP0619-L22739	Exon 25	3681-3682; 34 nt after exon 25	TGGAAGCCAAAT-51 nt spanning oligo-GCAAATAAAGCC	0.6 kb
250 #		03849-L18072	Exon 26	3816-3817	TGAGGCACTGTA-CGGTCCTTGCAA	0.3 kb
	409	18170-L26175	Exon 27	4002-4003	ATCGGTTTGAGA-GATTGGTGGAAC	2.6 kb
148		18364-L23329	Exon 28	4196-4197	GCAGACTCCATG-CAGACTCTCTTC	0.3 kb
400 #		04072-L03709	Exon 29	4323-4324	CATCCTCTGATT-GGCAACATGTTA	13.0 kb
	147	02512-L01943	Exon 30	4390-4391	TGAGGAAAACCA-GCGGAACCTCCT	3.9 kb
	472	18038-L26174	Exon 31	NM_001042492.2: 4497-4498	TTCTGTAGGCAA-CTTGCCACTCCC	5.5 kb

Length (nt) P081	Length (nt) P082	SALSA MLPA probe	Exon[a]	Ligation site[b] NM_000267.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
166 #		02513-L01944	Exon 32	4534-4535	CATCGGTGCAGT-AGGAAGTGCCAT	0.7 kb
	391 #	18365-L23330	Exon 33	4732-4733	TGTGAAAAGCAA-CTTTGATGCAGC	1.3 kb
	166	02514-L01945	Exon 34	4816-4817	TCTTTCCTTCAT-AAGTGACGGCAA	1.3 kb
184 #		18367-L23332	Exon 35	4958-4959	CTTGCATACCTG-GGTCCTCCAGAG	3.4 kb
	197 #	18374-L26502	Exon 36	12 nt before exon 36	ATTACTCTGTTA-TTTTTCTTTTAG	30.3 kb
	444 Ø	04069-L03311	OMG gene; Exon 2	NM_002544.4: 1073-1074, within NF1 intron 36	GCAGACAGTGGA-CACCATTAACTC	0.6 kb
454 Ø		04075-L03310	OMG gene; Exon 2	NM_002544.4: 507-506 reverse, within NF1 intron 36	CACAGAGACCGA-GGTAAGTGAGCA	30.1 kb
214		02517-L26127	Exon 37	5427-5428	GCCTCAAAGGTA-GCAAAAGGCTTG	1.5 kb
	211	02518-L01949	Exon 38	5720-5721	AACCAGTTCACC-TTAACCATTGCA	2.7 kb
238		02519-L01950	Exon 39	6008-6009	CTAGAGACATCA-GGTTTATGTATC	4.5 kb
	241	02520-L26200	Exon 40	6178-6179	GACTCCATGGCT-GTCAAATCTAGT	1.5 kb
272		02521-L22646	Exon 41	6390-6391	CAGGTGGCTTGG-GATCAATAAAAG	0.4 kb
436		03853-L29796	Exon 42	6592-6593	GCTGTCCTTCAA-CAATTCCCTTGA	0.7 kb
	419	03854-L23156	Exon 43	6800-6801	TCATTACCCAAA-TTTTACTTGCTG	0.4 kb
	249	12021-L26199	Exon 44	6989-6990	ATTCCAACGTGC-AAGTGGCTGGAC	0.2 kb
	454	03856-L03307	Exon 45	7075-7076	TCTTGTTGTCTT-TGGGTGTATTAG	0.6 kb
	265	02522-L01953	Exon 46	7162-7163	TTGCTTAAAAGG-ACCTGACACTTA	1.8 kb
289		04071-L01954	Exon 47	7269-7270	AAGCCCTCTTTT-GGGTAGCTGTGG	2.5 kb
	300	02524-L22720	Exon 48	7425-7426	ATCCTCTGGAGT-GGCACTGCAAGC	6.1 kb
319		02525-L22650	Exon 49	7535-7536	TCACCTGCTATT-GTTGCAAGAACA	1.0 kb
346		02526-L01957	Exon 50	7671-7672	AAGAAGTTCGAA-GTCGCTGCAGCC	2.1 kb
	328	13217-L22725	Exon 51	7799-7800	GAGACTCAGCCA-TGGTCCTCTCCC	0.1 kb
226		19363-L25737	Exon 51	7860-7861	CTGTCGGCCAGA-CCAGTCCCCGAG	4.2 kb
373		02528-L01959	Exon 52	7997-7998	AGGCAAGAAATG-GAATCAGGGATC	0.5 kb
	154	12018-L12866	Exon 53	8107-8108	TTTACGTAAAGT-TTCAGTGTCTGA	0.3 kb
	307	18034-L22721	Exon 54	8229-8230	AGTTTGATCAAC-GAATTCTTTATG	1.3 kb
	345	02529-L01960	Exon 55	8386-8387	GCAGAGTGTGGT-GTACCATGAAGA	0.4 kb
	382	18035-L22401	Exon 56	2 nt before exon 56	TTGATTTGTTGC-AGGTTTTGGTTT	1.6 kb
154		05220-L03309	Exon 57	8563-8564	TGGAATTGATGA-AGAAACCAGTGA	13.5 kb
391 «		02530-L01961	Exon 58	8748-8749	GCCACTGTAACA-GTGGACGAACTC	2.0 kb
208 «		19361-L26126	Exon 58	10796-10797	AGTGCCAAGGAT-GCCAAGCTGCCA	6.4 kb
136 « ¬		18363-L23328	Downstream	4.8 kb after exon 58	GGGAAGGAGCTC-AGGCTGTAATGT	
			stop codon	*8838-8840 (ex 58)*		

(a) The exon numbering used in this P081-D1/P082-C2 NF1 product description is the exon numbering from the NCBI NG_009018.1 which is identical to the LRG_214 sequence. The exon numbering and NM sequence used is from 04/2018, but can be changed (e.g. by NCBI) after the release of the product description.
(b) Ligation sites of the P081/P082 NF1 MLPA probes are indicated according to RefSeq sequence NM_000267.3 containing 57 exons, with the exception of the probe for exon 31. Ligation site of the last mentioned probe is indicated according to RefSeq sequence NM_001042492.2, containing 58 exons.
(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

« 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 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Probe detects the *OMG* gene, located within intron 36 of *NF1* gene. We have no information on the clinical significance of copy number changes of only the *OMG* gene.

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.

Related SALSA MLPA probemixes

P044 NF2:	Contains probes for the *NF2* gene, involved in Neurofibromatosis type 2.
P122 NF1 area:	Contains probes for the 17q11.2 region, involved in Neurofibromatosis type 1.
P295 SPRED1:	Contains probes for the *SPRED1* gene at 15q14, involved in Neurofibromatosis type 1-like syndrome.

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 P081/P082 NF1

- Ben-Salem S et al. (2014). The mutational spectrum of the NF1 gene in neurofibromatosis type I patients from UAE. *Childs Nerv Syst.* 30:1183-1189.
- Bendova S et al. (2007). Novel mutations in the NF1 gene in Czech patients with neurofibromatosis type 1. *J Mol Neurosci.* 31:273-279.
- De Luca A et al. (2007). Deletions of NF1 gene and exons detected by multiplex ligation-dependent probe amplification. *J Med Genet.* 44:800-808.
- Griffiths S et al. (2007). Molecular diagnosis of neurofibromatosis type 1: 2 years experience. *Fam Cancer.* 6:21-34.
- Imbard A et al. (2015). NF1 single and multi-exons copy number variations in neurofibromatosis type 1. *J Hum Genet.* 60:221-224.
- Laczmanska I et al. (2014). Exonic deletions in the NF1 gene in patients with neurofibromatosis type I from the lower Silesian region of Poland. *Adv Clin Exp Med.* 23:517-521.
- Mantripragada KK et al. (2006). Identification of novel deletion breakpoints bordered by segmental duplications in the NF1 locus using high resolution array-CGH. *J Med Genet.* 43:28-38.
- Maruoka R et al. (2014). The use of next-generation sequencing in molecular diagnosis of neurofibromatosis type 1: a validation study. *Genet Test Mol Biomarkers.* 18:722-735.
- Nemethova M et al. (2013). Thirty-nine novel neurofibromatosis 1 (NF1) gene mutations identified in Slovak patients. *Ann Hum Genet.* 77:364-379.
- Pasmant E et al. (2011). Role of noncoding RNA ANRIL in genesis of plexiform neurofibromas in neurofibromatosis type 1. *J Natl Cancer Inst.* 103:1713-1722.
- Sabbagh A et al. (2013). NF1 molecular characterization and neurofibromatosis type I genotype-phenotype correlation: the French experience. *Hum Mutat.* 34:1510-1518.
- Shen MH et al. (2007). Detection of copy number changes at the NF1 locus with improved high-resolution array CGH. *Clin Genet.* 72:238-244.
- Steinemann D et al. (2010). Mitotic recombination and compound-heterozygous mutations are predominant NF1-inactivating mechanisms in children with juvenile myelomonocytic leukemia and neurofibromatosis type 1. *Haematologica.* 95:320-323.
- Thomas L et al. (2012). Exploring the somatic NF1 mutational spectrum associated with NF1 cutaneous neurofibromas. *Eur J Hum Genet.* 20:411-419.
- Upadhyaya M et al. (2006). The heterogeneous nature of germline mutations in NF1 patients with malignant peripheral serve sheath tumours (MPNSTs). *Hum Mutat.* 27:716.
- Upadhyaya M et al. (2008). Germline and somatic NF1 gene mutations in plexiform neurofibromas. *Hum Mutat.* 29:E103-111.
- Upadhyaya M et al. (2009). The spectrum of somatic and germline NF1 mutations in NF1 patients with spinal neurofibromas. *Neurogenetics.* 10:251-263.

▪ Valero MC et al. (2011). A highly sensitive genetic protocol to detect NF1 mutations. *J Mol Diagn.* 13:113-122.
▪ van Minkelen R et al. (2014). A clinical and genetic overview of 18 years neurofibromatosis type 1 molecular diagnostics in the Netherlands. *Clin Genet.* 85:318-327.
▪ van Veghel-Plandsoen MM et al. (2011). Multiplex ligation-depending probe amplification is not suitable for detection of low-grade mosaicism. *Eur J Hum Genet.* 19:1009-1012.
▪ Wimmer K et al. (2006). Spectrum of single- and multiexon NF1 copy number changes in a cohort of 1,100 unselected NF1 patients. *Genes Chromosomes Cancer.* 45:265-276.
▪ Zhang J et al. (2015). Molecular Characterization of NF1 and Neurofibromatosis Type 1 Genotype-Phenotype Correlations in a Chinese Population. *Sci Rep.* 5:11291.

P081 Product history	
Version	*Modification*
D1	Exon 21 and exon 23 probes have been replaced, a probe for exon 21 has been added, a reference probe has been replaced, and several probes have a small change in length.
C1	Eleven target probes have been added or replaced and ten references have been added or replaced.
B2	Control fragments have been adjusted.
B1	Three NF1 probes and two reference probes have been replaced and two new control fragments at 100-105 nt have been included.
A1	First release.

P082 Product history	
Version	*Modification*
C2	Two reference probes have been replaced and one probe has a small change in length.
C1	Thirteen target probes have been added or replaced and eight references have been added or replaced.
B2	Control fragments have been adjusted.
B1	Six NF1 probes and five reference probes have been replaced and two new control fragments at 100-105 nt have been included.
A2	One reference probe has been replaced.
A1	First release.

Implemented changes in the product description

Version D1/C2-02 – 03 July 2018 (04)
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Information about Positive control DNA samples was adjusted.
- Product description adapted to a new template.
- Minor textual and layout changes.

Version P081-D1/P082-C2-01 – 06 July 2017 (03)
- 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).
- Remarks about variability and SNPs removed from information at Table 1.

Version 30 – 06 June 2017 (55)
- Product description adapted to a new product lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Information about NF1 gene and common microdeletion adjusted on page 1.
- Minor textual and layout changes.

Version 29 – 31 July 2015 (54)
- Warnings added in Table 1 and Table 2. Low signals for probes that consist of three parts can be due to depurination of the sample DNA.

Version 28 (53)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- New references added on page 1.

Version 27 (53)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 26 (53)
- Warning added to NF1 probe 02506-L02619 under table 1 and 2. This probe is more sensitive to experimental variation, due to a less stable binding of the probe to the DNA.

Version 25 (48)
- Warning added to NF1 probe 02521–L01952 under table 1. This probe is more sensitive to experimental variation, due to a less stable binding of the probe to the DNA.

Version 24 (48)
- Warning added below Table 1b and 2, probe 178 nt 02496-L01927.

Version 23 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 22 (48)
Product description adapted to a new version (lot number added, new pictures included, small textual changes, more reference articles added).

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