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
P082 version C2
For complete product history see page 11.

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 and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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.

Copy number variation (CNV) analysis of all exons of the *NF1* gene requires the use of both SALSA MLPA P081 NF1 mix 1 and SALSA MLPA P082 NF1 mix 2 probemixes.

Intended purpose
The SALSA MLPA Probemixes P081 NF1 mix 1 and P082 NF1 mix 2 are in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assays[2] for the detection of deletions or duplications in the *NF1* gene in genomic DNA isolated from human peripheral whole blood specimens. P081 NF1 mix 1 and P082 NF1 mix 2 are intended to confirm a potential cause for and clinical diagnosis of Neurofibromatosis type 1 (NF1) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P081 NF1 mix 1 and P082 NF1 mix 2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *NF1* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1]Please note that these probemixes are for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the products are for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

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 SALSA MLPA Probemix P044 NF2 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. Partial deletions and duplications 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 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 SALSA MLPA Probemix P122 NF1-area (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 ~283 kilobases (kb) on chromosome 17q11.2 and contains 58 exons. The *NF1* LRG_214 is available at www.lrg-sequence.org and is identical to GenBank NG_009018.1.

Transcript variants
For *NF1,* multiple transcript variants have been described. Transcript variant 2 lacks an in-frame coding exon compared to transcript variant 1 and encodes isoform 2 (NM_000267.3; 12381 nt; coding sequence 384-8840; http://www.ncbi.nlm.nih.gov/gene/4763). *NF1* transcript variant 1 (NM_001042492.3) represents the longest transcript and contains an additional in-frame coding exon (31).

Exon numbering
The *NF1* exon numbering used in this P081-D1/P082-C2 NF1 product description is the exon numbering from the LRG_214 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The P081 and P082 probemixes together contain at least 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 SALSA MLPA Probemix P081-D1 NF1 mix 1 contains 46 MLPA probes with amplification products between 130 and 463 nucleotides (nt), including 11 reference probes that detect autosomal chromosomal locations. The SALSA MLPA Probemix P082-C2 NF1 mix 2 contains 44 MLPA probes with amplification products between 130 and 483 nt, including nine reference probes that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

Both probemixes contain 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, 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.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.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 human peripheral whole blood specimens, 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
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. 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 different 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 (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics
The expected percentage 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 2006-2021 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms 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 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.mrcholland.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 each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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 (as described for *DMD* by 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 or flanking probes are unlikely to have any relation to the condition tested for.

P081/P082 specific notes:

- Due to the presence of pseudogenes, probe design in the *NF1* gene is difficult. The P081/P082 probemixes were designed to be specific for the *NF1* gene. This specificity was confirmed by testing of a positive sample with an *NF1* deletion. In rare cases, if changes in the pseudogenes occur, apparent duplications might be detected.
- Mosaicism has been reported in individuals with NF1. 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. CNV junction-specific long-range PCR, digital PCR or qPCR may be suitable for confirmation of low-level mosaic CNVs (Kluwe et al. 2020, Liu et al. 2020).

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *NF1* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemixes P081 NF1 mix 1/P082 NF1 mix 2.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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. Low-level mosaic CNVs may be confirmed using CNV junction-specific long-range PCR, digital PCR or qPCR (Kluwe et al. 2020, Liu et al. 2020).

***NF1* mutation database**

We strongly encourage users to deposit positive results in the LOVD Database (https://databases.lovd.nl/shared/genes/NF1). 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 SNVs and unusual results (e.g., a duplication of *NF1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.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	5q	
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	8q	
178	**NF1 probe** 02865-L02617		**Exon 4**
184	**NF1 probe** 18367-L23332		**Exon 35**
190	Reference probe 09836-L10246	11q	
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	10q	
272	**NF1 probe** 02521-L22646		**Exon 41**
279	Reference probe 12437-L13438	14q	
289	**NF1 probe** 04071-L01954		**Exon 47**
298	**NF1 probe** 02503-L22647		**Exon 13**
304	Reference probe 16436-L18889	18q	
312	**NF1 probe** 04076-L22649		**Exon 15**
319	**NF1 probe** 02525-L22650		**Exon 49**
328	Reference probe 05388-L04785	12p	
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	2p	
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	9q	
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	2q	
454 Ø	**OMG probe** 04075-L03310		**Intron 36 of *NF1* (*OMG* gene)**
463	Reference probe 09908-L10321	16p	

[a] See section Exon numbering on page 2 for more information.

* New in version D1.
¥ Changed in version D1. Minor alteration, 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. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ 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 the *NF1* gene. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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	5q	
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	16q	
184	Reference probe 17862-L22121	19q	
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	22q	
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	6q	
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	10p	
362 Ж	**NF1 probe** 18174-SP0619-L22739		**Exon 25**
372	Reference probe 08893-L23475	14q	
382	**NF1 probe** 18035-L22401		**Exon 56**
391	**NF1 probe** 18365-L23330		**Exon 33**
400 *	Reference probe 07808-L23525	3p	
409	**NF1 probe** 18170-L26175		**Exon 27**
419	**NF1 probe** 03854-L23156		**Exon 43**
427 Δ	**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**

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*NF1*
472	**NF1 probe** 18038-L26174		**Exon 31**
483 *	Reference probe 06676-L06254	11p	

[a] See section Exon numbering on page 2 for more information.

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
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. When this occurs in reference samples, it can look like an increased signal in the test samples.
Ø Probe detects the *OMG* gene, located within intron 36 of the *NF1* gene. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. NF1 probes arranged according to chromosomal location

Length (nt)		SALSA MLPA probe	*NF1* exon[a]	Ligation site NM_000267.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
P081	P082					
			start codon	*384-386 (Exon 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	598 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 # Δ	12025-L23157	Exon 19	3 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

Length (nt)		SALSA MLPA probe	*NF1* exon[a]	Ligation site NM_000267.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
P081	P082					
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	180 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; 35 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_001042492.3 4447-4448	TTCTGTAGGCAA-CTTGCCACTCCC	5.5 kb
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_002544.5: 950-951, within *NF1* intron 36	GCAGACAGTGGA-CACCATTAACTC	0.6 kb
454 Ø		04075-L03310	*OMG* gene; Exon 2	NM_002544.5: 384-383 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 (Exon 58)*		

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ 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 the *NF1* gene. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Complete probe sequences are available at www.mrcholland.com.

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 Legius syndrome.

References

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- 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.
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Selected publications using SALSA MLPA Probemixes P081/P082 NF1

- De Luca A et al. (2007). Deletions of NF1 gene and exons detected by multiplex ligation-dependent probe amplification. *J Med Genet.* 44:800-8.
- Giugliano T et al. (2019). Clinical and Genetic Findings in Children with Neurofibromatosis Type 1, Legius Syndrome, and Other Related Neurocutaneous Disorders. *Genes (Basel).* 10.
- Imbard A et al. (2015). NF1 single and multi-exons copy number variations in neurofibromatosis type 1. *J Hum Genet.* 60:221-4.
- Kang E et al. (2020). Phenotype categorization of neurofibromatosis type I and correlation to NF1 mutation types. *J Hum Genet.* 65:79-89.
- 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.* 18:722-35.
- Tsipi M et al. (2018). Phenotypic expression of a spectrum of Neurofibromatosis Type 1 (NF1) mutations identified through NGS and MLPA. *J Neurol Sci.* 395:95-105.
- Upadhyaya M et al. (2009). The spectrum of somatic and germline NF1 mutations in NF1 patients with spinal neurofibromas. *Neurogenetics.* 10:251-63.
- Valero MC et al. (2011). A highly sensitive genetic protocol to detect NF1 mutations. *J Mol Diagn.* 13:113-22.

- 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.* 19:1009-12.
- 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.* 45:265-76.

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/C2-08 – 25 March 2025 (04P)
- Warnings for salt sensitivity removed for the 391 nt probe (02530-L01961) and the 208 nt probe (19361-L26126) from Table 1a and Table 2.
- Warning for SNP rs562084304 removed for the 409 nt probe (08725-L08736) from Table 1a and been replaced by a general warning for small sequence changes, included in section Limitations of the procedure.
- List of selected publications shortened.

Version D1/C2-07 – 29 January 2024 (04P)
- Morocco removed from the list of countries in which the product is IVD registered.

Version D1/C2-06 – 31 January 2022 (04P)
- Added length of the Spanning Oligo to the 256 nt probe (18033-SP0601-L29798) in Table 2.
- Exon number corrected for the 454 nt probe (03856-L03307) in Table 1b, Table 2 and the Appendix.
- Minor textual and lay-out changes.

Version D1/C2-05 – 15 July 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended purpose updated.
- UK has been added to the list of countries in Europe that accept the CE mark.
- Added possible methods to confirm mosaic CNV results to the Probemix-specific notes and Confirmation of results section.
- Ligation sites of the probes targeting the NF1 gene updated according to new version of the NM_ reference sequence (NM_001042492.3).

- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene (166 nt probe 02514-L01945).
- Warning added to Table 1a for SNP that could influence the probe signal of Reference probe 08725-L08736
- Four recent articles added to the list of Selected publications, one article removed.

Version D1/C2-04 – 22 April 2020 (04)
- Product is now registered for IVD use in Colombia.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

IVD	EUROPE* CE ISRAEL COLOMBIA
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.

Appendix I: Additional information on *NF1* exon numbering

The table below describes the *NF1* exon numbering and ligation sites, based on NM_000267.3 and NM_001042492.3.

Length (nt)		SALSA MLPA probe	*NF1* exon based on **LRG_214**	*NF1* exon numbering and ligation site based on **NM_000267.3**		*NF1* exon numbering and ligation site based on **NM_001042492.3**	
P081	**P082**			*start codon*	*384-386 (Exon 1)*	*start codon*	*334-336 (Exon 1)*
196 ¬		18368-L23333	**Upstream**	**Upstream**	8.0 kb before exon 1	**Upstream**	8.1 kb before exon 1
142		02491-L29974	**Exon 1**	**Exon 1**	335 nt before exon 1	**Exon 1**	385 nt before exon 1
	227	19362-L26201	**Exon 1**	**Exon 1**	53-54	**Exon 1**	3-4
	138	18382-L19008	**Exon 1**	**Exon 1**	415-414 reverse	**Exon 1**	365-364 reverse
	190	12019-L12867	**Intron 1**	**Intron 1**	598 nt after exon 1	**Intron 1**	598 nt after exon 1
160		02493-L01924	**Exon 2**	**Exon 2**	475-476	**Exon 2**	425-426
	160	02494-L01925	**Exon 3**	**Exon 3**	631-632	**Exon 3**	581-582
178		02865-L02617	**Exon 4**	**Exon 4**	735-736	**Exon 4**	685-686
	172	18173-L22738	**Exon 5**	**Exon 5**	958-959	**Exon 5**	908-909
202		02497-L03706	**Exon 6**	**Exon 6**	1000-1001	**Exon 6**	950-951
220		18032-L22398	**Exon 7**	**Exon 7**	1046-1047	**Exon 7**	996-997
	205	02498-L22716	**Exon 8**	**Exon 8**	1167-1168	**Exon 8**	1117-1118
	463 Ж #	18037-SP0602-L22403	**Exon 9**	**Exon 9**	1341-1342; 1372-1373	**Exon 9**	1291-1292; 1322-1323
	233 #	02500-L26202	**Exon 10**	**Exon 10**	1508-1509	**Exon 10**	1458-1459
232 #		13221-L26128	**Exon 11**	**Exon 11**	291 nt after exon 11	**Exon 11**	291 nt after exon 11
	257	03778-L26198	**Exon 12**	**Exon 12**	1722-1723	**Exon 12**	1672-1673
298 #		02503-L22647	**Exon 13**	**Exon 13**	1849-1850	**Exon 13**	1799-1800
	292	02504-L26817	**Exon 14**	**Exon 14**	1985-1986	**Exon 14**	1935-1936
312		04076-L22649	**Exon 15**	**Exon 15**	235 nt before exon 15 reverse	**Exon 15**	235 nt before exon 15 reverse
	281 Ж #	19364-SP0809-L25738	**Exon 15**	**Exon 15**	2053-2054; 2083-2084	**Exon 15**	2003-2004; 2033-2034
	317 Ж #	18369-SP0646-L23334	**Exon 16**	**Exon 16**	35 nt before exon 16; 2107-2108	**Exon 16**	35 nt before exon 16; 2057-2058
353		02507-L22658	**Exon 17**	**Exon 17**	2329-2330	**Exon 17**	2279-2280
427 #		12024-L26426	**Exon 18**	**Exon 18**	2587-2588	**Exon 18**	2537-2538
	427 # Δ	12025-L23157	**Exon 19**	**Exon 19**	3 nt after exon 19	**Exon 19**	3 nt after exon 19
	337	18370-L23335	**Exon 20**	**Exon 20**	34 nt before exon 20	**Exon 20**	34 nt before exon 20
244 #		21185-L29794	**Exon 21**	**Exon 21**	3064-3063 reverse	**Exon 21**	3014-3013 reverse
382 #		21186-L29799	**Exon 21**	**Exon 21**	3170-3171	**Exon 21**	3120-3121
	436 #	18036-L22765	**Exon 22**	**Exon 22**	3269-3270	**Exon 22**	3219-3220
418 Ж #		18408-SP0653-L23405	**Exon 23**	**Exon 23**	3434-3435; 3468-3469	**Exon 23**	3384-3385; 3418-3419
337		21000-L29222	**Exon 23**	**Exon 23**	180 nt after exon 23	**Exon 23**	180 nt after exon 23
256 Ж #		18033-SP0601-L29798	**Exon 24**	**Exon 24**	37 nt before exon 24; 3498-3499	**Exon 24**	37 nt before exon 24; 3448-3449
	362 Ж	18174-SP0619-L22739	**Exon 25**	**Exon 25**	3681-3682; 35 nt after exon 25	**Exon 25**	3631-3632; 35 nt after exon 25
250 #		03849-L18072	**Exon 26**	**Exon 26**	3816-3817	**Exon 26**	3766-3767
	409	18170-L26175	**Exon 27**	**Exon 27**	4002-4003	**Exon 27**	3952-3953
148		18364-L23329	**Exon 28**	**Exon 28**	4196-4197	**Exon 28**	4146-4147
400 #		04072-L03709	**Exon 29**	**Exon 29**	4323-4324	**Exon 29**	4273-4274
	147	02512-L01943	**Exon 30**	**Exon 30**	4390-4391	**Exon 30**	4340-4341
	472	18038-L26174	**Exon 31**	**Intron 30**	3.8 kb after exon 30	**Exon 31**	4447-4448
166 #		02513-L01944	**Exon 32**	**Exon 31**	4534-4535	**Exon 32**	4547-4548
	391 #	18365-L23330	**Exon 33**	**Exon 32**	4732-4733	**Exon 33**	4745-4746
	166 #	02514-L01945	**Exon 34**	**Exon 33**	4816-4817	**Exon 34**	4829-4830

Length (nt)		SALSA MLPA probe	*NF1* exon based on **LRG_214**	*NF1* exon numbering and ligation site based on **NM_000267.3**		*NF1* exon numbering and ligation site based on **NM_001042492.3**	
P081	**P082**						
184 #		18367-L23332	**Exon 35**	**Exon 34**	4958-4959	**Exon 35**	4971-4972
	197 #	18374-L26502	**Exon 36**	**Exon 35**	12 nt before exon 35	**Exon 36**	12 nt before exon 36
	444 Ø	04069-L03311	***OMG* gene; Exon 2**	***OMG* gene; Exon 2**	NM_002544.5: 950-951, within *NF1* intron 35	***OMG* gene; Exon 2**	NM_002544.5: 950-951, within *NF1* intron 36
454 Ø		04075-L03310	***OMG* gene; Exon 2**	***OMG* gene; Exon 2**	NM_002544.5: 384-383 reverse, within *NF1* intron 35	***OMG* gene; Exon 2**	NM_002544.5: 384-383 reverse, within *NF1* intron 36
214		02517-L26127	**Exon 37**	**Exon 36**	5427-5428	**Exon 37**	5440-5441
	211	02518-L01949	**Exon 38**	**Exon 37**	5720-5721	**Exon 38**	5733-5734
238		02519-L01950	**Exon 39**	**Exon 38**	6008-6009	**Exon 39**	6021-6022
	241	02520-L26200	**Exon 40**	**Exon 39**	6178-6179	**Exon 40**	6191-6192
272		02521-L22646	**Exon 41**	**Exon 40**	6390-6391	**Exon 41**	6403-6404
436		03853-L29796	**Exon 42**	**Exon 41**	6592-6593	**Exon 42**	6605-6606
	419	03854-L23156	**Exon 43**	**Exon 42**	6800-6801	**Exon 43**	6813-6814
	249	12021-L26199	**Exon 44**	**Exon 43**	6989-6990	**Exon 44**	7002-7003
	454	03856-L03307	**Exon 45**	**Exon 44**	7075-7076	**Exon 45**	7088-7089
	265	02522-L01953	**Exon 46**	**Exon 45**	7162-7163	**Exon 46**	7175-7176
289		04071-L01954	**Exon 47**	**Exon 46**	7269-7270	**Exon 47**	7282-7283
	300	02524-L22720	**Exon 48**	**Exon 47**	7425-7426	**Exon 48**	7438-7439
319		02525-L22650	**Exon 49**	**Exon 48**	7535-7536	**Exon 49**	7548-7549
346		02526-L01957	**Exon 50**	**Exon 49**	7671-7672	**Exon 50**	7684-7685
	328	13217-L22725	**Exon 51**	**Exon 50**	7799-7800	**Exon 51**	7812-7813
226		19363-L25737	**Exon 51**	**Exon 50**	7860-7861	**Exon 51**	7873-7874
373		02528-L01959	**Exon 52**	**Exon 51**	7997-7998	**Exon 52**	8010-8011
	154	12018-L12866	**Exon 53**	**Exon 52**	8107-8108	**Exon 53**	8120-8121
	307	18034-L22721	**Exon 54**	**Exon 53**	8229-8230	**Exon 54**	8242-8243
	345	02529-L01960	**Exon 55**	**Exon 54**	8386-8387	**Exon 55**	8399-8400
	382	18035-L22401	**Exon 56**	**Exon 55**	2 nt before exon 55	**Exon 56**	2 nt before exon 56
154		05220-L03309	**Exon 57**	**Exon 56**	8563-8564	**Exon 57**	8576-8577
391 «		02530-L01961	**Exon 58**	**Exon 57**	8748-8749	**Exon 58**	8761-8762
208 «		19361-L26126	**Exon 58**	**Exon 57**	10796-10797	**Exon 58**	10809-10810
136 « ¬		18363-L23328	**Downstr.**	**Downstr.**	4.8 kb after exon 57	**Downstr.**	4.8 kb after exon 58
				stop codon	*8838-8840 (Exon 57)*	*stop codon*	*8851-8853 (Exon 58)*

Probes for which exon numbering between the NM sequences is different are marked in grey.

Note 1: The exon numbering and the NM_ sequences used have been retrieved on 06/2021. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Note 2: See page 9 and 10 for a description of the warnings (¬, Ж, #, Δ, Ø, «).