Product Description SALSA® MLPA® Probemix P044-C1 NF2

To be used with the MLPA General Protocol.

Version C1. As compared to version B3, nine new target probes have been included, one target probe has been replaced, and one target probe has been removed. In addition, ten reference probes have been replaced and the total number of reference probes has been increased to 13. Several probes have changed in length but not in the sequence detected. For complete product history see page 9.

Catalogue numbers:
- **P044-025R:** SALSA MLPA Probemix P044 NF2, 25 reactions.
- **P044-050R:** SALSA MLPA Probemix P044 NF2, 50 reactions.
- **P044-100R:** SALSA MLPA Probemix P044 NF2, 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.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 P044 NF2 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the *NF2* gene in order to confirm a potential cause and clinical diagnosis for Neurofibromatosis type 2 (NF2). This product can also be used for molecular genetic testing of at-risk family members.

This assay is for use with human DNA extracted from peripheral blood. In a research setting this assay can be used on DNA derived from fresh or formalin-fixed paraffin-embedded (FFPE) tumour tissue. Deletions or duplications detected with the P044 NF2 probemix 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 aforementioned gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of this gene. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test must be interpreted by a clinical molecular geneticist or equivalent. NF2 has a high incidence of mosaicism: ~15% of NF2 patients are mosaic. Mosaic mutations may not be detectable in blood or other healthy tissues.

[1]Please note that this probemix is for In Vitro Diagnostic (IVD) use 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 type 2 (NF2) is an autosomal dominant cancer syndrome that is characterized by the development of bilateral vestibular schwannomas (BVSs) in almost all patients. This disease is caused by inactivating mutations of the *NF2* tumour-suppressor gene. BVSs result in hearing loss, tinnitus and balance dysfunction. The age of onset is between 18 and 24 years. Patients also suffer from schwannomas of other cranial and peripheral nerves, meningiomas, ependymomas, and rarely, astrocytomas. Although most of these tumours are not malignant, their anatomic location and multiplicity lead to high morbidity and mortality at a low age: the average age of death is 36 years. NF2 occurs in approximately 1 in 25,000-40,000 live births and the estimated prevalence in the general population is 1 in 60,000 without any known ethnic or racial bias. For known pathogenic mutations the penetrance is close to 100% (Asthagiri et al. 2009).

Mutational analysis of the *NF2* gene in typical NF2 patients has demonstrated causative mutations in ~70%. The *NF2* gene behaves as a typical tumour-suppressor gene, with first hits detectable in both constitutional and tumour specimens and second hits detectable only in tumours. Approximately 50% of *NF2* mutation positive patients inherit a germline mutation from an affected parent and the remaining half are sporadic cases due to *de novo* mutations. Large alterations affecting the *NF2* gene account for 15-20% of all known *NF2* mutations (Abo-Dalo et al. 2010; Halliday et al. 2017; Kluwe et al. 2005; Smith et al. 2016). Combined with the 70% *NF2* mutation detection rate in patients this means that 10-15% of NF2 patients have large deletions or duplications. A high level of mosaicism is observed in NF2, which can complicate mutation detection. More than 30% of the *de novo* cases are mosaic for *NF2* mutations, which may result in subclinical symptoms and/or difficulties with mutation detection, resulting in a false negative diagnosis (Evans et al. 2007).

Mutations in *NF2* are also frequently found in the DNA of sporadic schwannomas and meningiomas (Lassaletta et al. 2013; Mohyuddin et al. 2002; Pathmanaban et al. 2017). Both NF2 syndromic tumours and such sporadic tumours have often lost a large part of chromosome 22 resulting in loss of heterozygosity (LOH) of *NF2*. These large chromosomal deletions frequently include loss of *SMARCB1* and *LZTR1*, which are also recognized as tumour suppressor genes associated with an NF2-related disorder: schwannomatosis.

More information on NF2 can be found at https://www.ncbi.nlm.nih.gov/books/NBK1201/ and https://omim.org/entry/101000.

Gene structure: The *NF2* gene has 17 exons and spans 95 kb of genomic DNA on chromosome 22q12, 21 Mb from the q-telomere. The *NF2* LRG_511 is identical to NG_009057.1 and is available at: www.lrg-sequence.org.

Transcript variants: There are multiple *NF2* transcripts listed by NCBI but only transcript variants 1 and 2 are reference standards in the RefSeqGene project: variant 1, NM_000268.3, is 6046 nt long with coding sequence 444-2231 and variant 2, NM_016418.5 is 6091 nt long with coding sequence 444-2216. These two transcript are also the only transcripts listed under the LRG_511. The difference between the two transcripts is that NM_016418.5 contains all 17 exons, while NM_000268.3 lacks exon 16. In Table 2, the ligation sites of the *NF2* probes are indicated according to the predominant variant NM_000268.3.

Exon numbering: The *NF2* exon numbering used in this P044-C1 NF2 product description is the exon numbering from the LRG_511 sequence. The exon numbering used has been retrieved in September 2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P044-C1 NF2 contains 43 MLPA probes with amplification products between 129 and 472 nucleotides (nt). This includes 21 probes for the *NF2* gene; 5 probes on chromosome 22q upstream of *NF2*, 4 of which target *SMARCB1* and *LZTR1*; and 4 probes on chromosome 22q downstream of *NF2*. In addition, 13 reference probes are included that detect autosomal chromosomal targets that have stable copy numbers in the general population and have relatively stable copy numbers in various cancer types including schwannomas and meningiomas. Complete probe sequences and the identity of the genes detected by the reference probes are 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, 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.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 nt and 96 nt 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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 blood is necessary for in vitro diagnostic use. In a research setting, DNA derived from fresh or FFPE tumour tissue, free from impurities known to affect MLPA reactions may be used. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 healthy individuals. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Please note that when analysing DNA from FFPE tumour material, the required reference samples are DNA from healthy FFPE tissue material, isolated using the same method. Using unmatched reference samples to analyse DNA from FFPE tumour material has a high chance of false positives.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number(s) NA07106 from the Coriell Institute has been tested with this P044-C1 probemix at MRC-Holland and can be used as a positive control sample to detect a heterozygous duplication of the entire *NF2* gene including flanking regions targeted by the target probes present in this probemix. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics: In ~70% of patients with a clinical diagnosis of NF2 a germline mutation in the *NF2* gene is identified, and 15-20% of all known pathogenic *NF2* mutations are large deletions or duplications. Therefore, the minimal diagnostic sensitivity of P044-C1 is estimated at 10-15%. Analytical performance for the detection of deletions/duplications in *NF2* is very high and can be considered >99% (based on a 2005-2018 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 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.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.

Note: The slope correction algorithm in Coffalyser.Net has been optimised to give the best possible results in as many situations as possible. However, the slope correction algorithm may confuse a large deletion for sloping, which can lead to over- or under-correction. Since large deletions are often detected when using the P044 probemix, this issue may occur relatively frequently. Incorrectly applied slope correction can cause an FSLP warning in Coffalyser.Net or ambiguous results for multiple probes. If you suspect that slope correction was incorrectly applied we recommend to contact info@mlpa.com for assistance.

Interpretation of results: The expected results for the *NF2* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 0 (homozygous deletion) or 4 (homozygous duplication or heterozygous triplication). The standard deviation of each individual probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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

Please note that these above mentioned dosage quotients are only valid for germline testing. For tumour derived samples dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- 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. 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net

software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

P044 specific note:

- The P044 probemix contains five probes with targets on 22q upstream of the *NF2* gene and four probes with targets on 22q downstream of the *NF2* gene. Four of the upstream probes target two other tumour suppressor genes: *LZTR1* and *SMARCB1*. LOH of *NF2* in tumours (sporadic or NF2-associated) often also involves loss of *LZTR1* and *SMARCB1*. The flanking probes are included to determine the extent of the deletion on chromosome 22q. *SMARCB1* is associated with schwannomatosis 1 and *LZTR1* is associated with schwannomatosis 2. Schwannomatosis is a tumour predisposition syndrome related to NF2.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *NF2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P044 NF2.
- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in tumours with high chromosomal instability.

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.

***NF2* mutation database:** https://databases.lovd.nl/shared/genes/NF2 is used for germline *NF2* mutations. Somatic *NF2* mutations found in cancer can be found in the COSMIC database: https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=NF2/ . We strongly encourage users to deposit positive results in the appropriate 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 *NF2* exons 3 and 5 but not exon 4) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P044-C1 NF2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*NF2*
64-105	Control fragments – see table in probemix content section for more information		
129 ¥	Reference probe 18709-L26847	5q31	
136 *	**NF2** probe 22444-L31624		**Exon 17**
143 ¥	**NF2** probe 01563-L31617		**Upstream**
148 *	Reference probe 14199-L23450	2q13	
154 *	**NF2** probe 22445-L31625		**Exon 9**
158 ¥	**NF2** probe 22439-L31614		**Exon 13**
166 *	Reference probe 16253-L31432	19p13	
172 ¥ ±	**NF2** probe 01565-L31618		**Exon 2**
178 ¥	**NF2** probe 01566-L31619		**Exon 3**
184 *	Reference probe 16915-L19859	9q22	
190 ¥	**NF2** probe 22440-L31615		**Exon 15**
197 ¥	**NF2** probe 01567-L31620		**Exon 4**
203 *	Reference probe 13121-L31675	16p11	
208 ¥	**NF2** probe 01568-L31621		**Exon 5**
217 *	**NF2** probe 22446-L31626		**Exon 17**
226	**NF2** probe 18696-L29634		**Exon 7**
236 *	Reference probe 19652-L26684	4p13	
244 ¥	**NF2** probe 22441-L04978		**Exon 1**
254 ¥	**NF2** probe 01571-L31622		**Exon 8**
263 *	Reference probe 08812-L24457	2p13	
270 ¥	**NF2** probe 22442-L02031		**Exon 6**
280	**NF2** probe 15774-L17826		**Exon 11**
292 *	**EP300** probe 22449-L31628		**Telomeric**
301 *	Reference probe 17452-L21208	12p13	
310 ¥	**NF2** probe 01575-L31623		**Exon 12**
319 * +	**SMARCB1** probe 08294-L20837		**Centromeric**
328	**NF2** probe 01577-L01149		**Exon 14**
337 ¥	**NF2** probe 22443-L31616		**Exon 10**
346 *	Reference probe 04337-L20895	15q21	
355 ๑	**NF2** probe 03318-L02736		**Exon 16**
366	**NF2** probe 01580-L29633		**Exon 17**
374 *	Reference probe 10718-L31856	6p12	
382 ±	**NF2** probe 01581-L01135		**Upstream**
391 « ¬	**NIPSNAP1** probe 02580-L02042		**Centromeric**
400 * +	**LZTR1** probe 22448-L27086		**Centromeric**
409 ¥¬	**CABP7** probe 03317-L31857		**Telomeric**
416 *	Reference probe 22471-L24662	4q22	
427 * ¬	**ALG12** probe 22450-L31629		**Telomeric**
433 * +	**SMARCB1** probe 22451-L25981		**Centromeric**
445 *	Reference probe 05916-L14204	21q11	
454 * +	**LZTR1** probe 20015-L27094		**Centromeric**
463 * ¬	**LARGE1** probe 12460-L13461		**Telomeric**
472 *	Reference probe 14846-L16554	3q11	

a) See above section on exon numbering for more information.

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in sequence detected.
± SNP rs1800538 could influence the probe signal of probe 01581-L01135 at 382nt. COSM21991 (equivalent to rs121434259) could influence the probe signal of probe 01565-L31618 at 172 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
๑ This sequence is not part of the main transcript NM_000268.3 that lacks exon 16, nor is it part of exon 16 in NM_016418.5, but it is located in the longer exon 16 of the alternative transcript NM_181825.2 (ligation

site 2622-2623). The significance of copy number changes detected by only this probe is not clear as it targets a non-coding part of the *NF2* gene.
¬ 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.
+ *SMARCB1* and *LZTR1* are tumour suppressor genes associated with schwannomatosis, which is a tumour predisposition syndrome related to NF2. Additionally, LOH of *NF2* in sporadic and NF2-associated tumours often includes loss of *SMARCB1* and *LZTR1*. These probes also help to determine the extent of the deletion/duplication on chromosome 22.

Table 2. *NF2* region probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*NF2* exon[a]	Ligation site NM_000268.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
454 +	20015-L27094	LZTR1		GCTCAATGACCT-CCTGCGGTTCGA	11.1 kb
400 +	22448-L27086	LZTR1		ATTGTGCACCAG-TTCACCAAGGTC	2778.1 kb
433 +	22451-L25981	SMARCB1		TGGCGCTGAGCA-AGACCTTCGGGC	46.5 kb
319 +	08294-L20837	SMARCB1		CTGAGATGGAGA-AGAAGATCCGCG	5775.9 kb
391 « ¬	02580-L02042	NIPSNAP1		AGGCTGACAAGT-TCTGAGGATTAC	47.4 kb
143	01563-L31617	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
		start codon	*444-446 (exon 1)*		
244	22441-L04978	Exon 1	487-488	CAGCTCTCTCAA-GAGGAAGCAACC	32.8 kb
172 ±	01565-L31618	Exon 2	628-629	AACCTGGTTCTT-TGGACTGCAGTA	2.3 kb
178	01566-L31619	Exon 3	759-760	CTGAGAATGCTG-AAGAGGAGCTGG	3.1 kb
197	01567-L31620	Exon 4	855-856	CCTCCTGAGGCT-TCTGTGCTCCTG	12.4 kb
208	01568-L31621	Exon 5	915-916	ACCCCAGTGTTC-ACAAGCGGGGAT	1.0 kb
270	22442-L02031	Exon 6	1002-1003	TGTGGGAGGAGA-GAATTACTGCTT	2.6 kb
226	18696-L29634	Exon 7	1070-1071	GAATATCTGAAG-ATAGCTCAGGAC	3.0 kb
254	01571-L31622	Exon 8	1171-1172	GGGGCTTCACAT-TTATGACCCTGA	3.7 kb
154	22445-L31625	Exon 9	1277-1278	CCACTGGATAAG-AAAATTGATGTC	3.4 kb
337	22443-L31616	Exon 10	1434-1433 reverse	CACCTGCTTTCT-AGCCTTCTCCTC	3.5 kb
280	15774-L17826	Exon 11	1546-1545 reverse	CGTTGGCCATTG-TTGCTTCTTCTT	1.4 kb
310	01575-L31623	Exon 12	1624-1625	CACCGAGGAGGA-GGCAAAACTTCT	1.5 kb
158	22439-L31614	Exon 13	1813-1814	GCTGAAGCAGGA-CCTGCAGGAAGC	3.4 kb
328	01577-L01149	Exon 14	1945-1946	CTTCAACCTCAT-TGGTGACAGCCT	3.2 kb
190	22440-L31615	Exon 15	2071-2072	CAATGAACTCAA-GACAGAAATCGA	2.0 kb
355 ◎	03318-L02736	Exon 16	1.9kb after exon 15; NM_181825.2; 2622-2623	CTGGTGTGTTTA-ACTCAAGATCAA	11.3 kb
136	22444-L31624	Exon 17	2219-2218 reverse	TAGAGCTCTTCA-AAGAAGGCCACT	0.1 kb
		stop codon	*2229-2231 (exon 17)*		
366	01580-L29633	Exon 17	2304-2305	CAGATATCAAGA-GAGCCATCCATA	3.3 kb
217	22446-L31626	Exon 17	5645-5644 reverse	GAAGTGTTATGT-AATGCCACGGGG	30.9 kb
409 ¬	03317-L31857	CABP7		ACATAGAGAACA-TCATCATGACGG	3655.0 kb
463 ¬	12460-L13461	LARGE1		AGGAATAGCTGC-ACCTTCGAACCT	7773.0 kb
292 ¬	22449-L31628	EP300		CTACCATTAAGA-GGAAGTTAGACA	8800.2 kb
427 ¬	22450-L31629	ALG12		TCCAGCACAATT-ATGACAATTCAG	

a) See above section on exon numbering for more information.
b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

± SNP rs1800538 could influence the probe signal of probe 01581-L01135 at 382nt. COSM21991 (equivalent to rs121434259) could influence the probe signal of probe 01565-L31618 at 172 nt. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.

ɷ This sequence is not part of the main transcript NM_000268.3 that lacks exon 16, nor is it part of exon 16 in NM_016418.5, but it is located in the longer exon 16 of the alternative transcript NM_181825.2 (ligation site 2622-2623). The significance of copy number changes detected by only this probe is not clear as it targets a non-coding part of the *NF2* gene.
¬ 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.
+ *SMARCB1* and *LZTR1* are tumour suppressor genes associated with schwannomatosis, which is a tumour predisposition syndrome related to NF2. Additionally, LOH of *NF2* in sporadic and NF2-associated tumours often includes loss of *SMARCB1* and *LZTR1*. These probes also help to determine the extent of the deletion/duplication on chromosome 22.

Related SALSA MLPA probemixes

- P081/P082 NF1: These two probemixes contain probes for all exons of the *NF1* gene, which is associated with Neurofibromatosis type I (NF1).
- P122 NF1 AREA: This probemix contains probes for the *NF1* region on chromosomal band 17q11.2.
- P258 SMARCB1: This probemix contains probes for all exons of the *SMARCB1* gene, which is associated with rhabdoid tumour development. The two *SMARCB1* probes in P044-C1 are also included in P258-C1.
- P294 Tumour loss: This probmix contains probe for fifteen chromosomal regions, which are frequently deleted in tumour samples. The *SMARCB1* gene is among these regions. The *SMARCB1* exon 1 probe in P044-C1 is also included is P294-C1.
- P455 LZTR1: This probemix contains probes for most exons of *LZTR1*, a gene associated with schwannomatosis. The two *LZTR1* probes in P044-C1 are also included in P455-A1.

References

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P044 Product history	
Version	*Modification*
C1	Nine new target probes have been included, one replaced, and one removed. Ten reference probes have been replaced and the total number of reference probes has been increased to 13. Several probes have changed in length but not in the sequence detected.
B3	Two reference probes were replaced and several probe lengths were adjusted.
B2	One reference probe was replaced. In addition, the 88 and 96 nt control fragments were replaced (QDX2).
B1	One *NF2* probe and six reference probes were replaced. In addition, four control fragments at 88-96-100-105 nt were included.
A1	First release.

Implemented changes in the product description
Version C1-01 – 01 October 2019 (02P) - Product description rewritten and adapted to a new template. - P044-C1 is now CE marked. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). *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 – 10 May 2013 (49)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

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RUO	ALL OTHER COUNTRIES

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