Product Description
SALSA® MLPA® Probemix P044-C1 NF2

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 11.

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.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.

Intended purpose
The SALSA MLPA Probemix P044 NF2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *NF2* gene in genomic DNA isolated from human peripheral whole blood specimens. P044 NF2 is intended to confirm a potential cause for and clinical diagnosis of *NF2*-related schwannomatosis and for molecular genetic testing of at-risk family members. *NF2*-related schwannomatosis has a high incidence of mosaicism (~15% of patients are mosaic) and mosaic mutations may not be detectable in blood.

Copy number variations (CNVs) detected with P044 NF2 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 *NF2* 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.

Only in a research setting this assay can be used on DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[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).

[2]To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background

NF2-related schwannomatosis (formerly known as Neurofibromatosis type 2) is an autosomal dominant cancer susceptibility/predisposition 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 neurofibromatosis type 2 (*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*-related schwannomatosis occurs in approximately 1 in 25,000-40,000 live births and the estimated prevalence in the general population is 1 in 50,000 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*-related schwannomatosis 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 in 10-15% of *NF2*-related schwannomatosis patients large deletions or duplications are detected. A high level of mosaicism is observed in *NF2*-related schwannomatosis, 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 sporadic schwannomas and meningiomas (Lassaletta et al. 2013; Mohyuddin et al. 2002; Pathmanaban et al. 2017). Both *NF2*-related schwannomatosis 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 schwannomatosis.

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

Gene structure

The *NF2* gene spans 95 kilobases (kb) on chromosome 22q12.2 and contains 17 exons. The *NF2* LRG_511 is available at www.lrg-sequence.org and is identical to GenBank NG_009057.1.

Transcript variants

For *NF2*, multiple transcript variants have been described. Transcript variants 1 and 2 are the only ones listed in LRG_511 for *NF2* gene. Transcript variant 1, NM_000268.4, is 5950 nt long with coding sequence 367-2154 and variant 2, NM_016418.5 is 6091 nt long with coding sequence 444-2216 (https://www.ncbi.nlm.nih.gov/gene/4771). The difference between the two transcripts is that NM_016418.5 contains all 17 exons, while NM_000268.4 lacks exon 16. In Table 2, the ligation sites of the *NF2* probes are indicated according to the NM_000268.4 (transcript variant 1).

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 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 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; five flanking probes on chromosome 22q in the centromeric region upstream of *NF2*, four of which target *SMARCB1* and *LZTR1*; and four flanking probes on chromosome 22q in the telomeric region 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/partial probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and online (www.mrcholland.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.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). 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, free from impurities known to affect MLPA reactions. In a research setting, DNA derived from fresh or FFPE tumour tissue 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 different unrelated individuals who are from families without a history of *NF2*-related schwannomatosis. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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 results.

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. Sample ID number 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 adjacent regions (all *NF2* region probes listed in Table 2). 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*-related schwannomatosis, 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 2005-2023 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 *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 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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios 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/subclonal 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. 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, unless otherwise specified (i.e. *SMARCB1* and *LZTR1*).
- 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: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P044 specific notes:

- The P044 probemix contains nine probes flanking the *NF2* gene, which are included to determine the extent of the CNV on chromosome 22q. Five of these nine probes target the upstream region of *NF2* (centromeric flanking probes) and four target the downstream region of NF2 (telomeric flanking probes). Four of the centromeric flanking probes target two other tumour suppressor genes: *LZTR1* and *SMARCB1*. LOH of *NF2* in tumours (sporadic or associated to NF2-related schwannomatosis) often also involves loss of *LZTR1* and *SMARCB1*. *SMARCB1* is associated with schwannomatosis 1 and *LZTR1* is associated with schwannomatosis 2. Schwannomatosis is a tumour predisposition syndrome related to *NF2*-related schwannomatosis.
- 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@mrcholland.com for assistance.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *NF2* gene are small (point) mutations, none 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. 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.

- 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 SNVs and unusual results (e.g., a duplication of *NF2* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P044-C1 NF2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*NF2*	Flanking
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 section Exon numbering on page 2 for more information.

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in sequence detected.
± 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.

¬ 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, unless indicated otherwise.

⍟ This sequence is not part of the predominant transcript NM_000268.4 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_181825.3 (ligation site 2545-2546). 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.

+ *SMARCB1* and *LZTR1* are tumour suppressor genes associated with schwannomatosis, which is a tumour predisposition syndrome related to *NF2*-related schwannomatosis. Additionally, LOH of *NF2* in sporadic and *NF2*-related schwannomatosis-associated tumours often includes loss of *SMARCB1* and *LZTR1*. These probes also help to determine the extent of the deletion/duplication on chromosome 22.

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. *NF2* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Location / Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Centromeric flanking probes.					
454 + ¬	20015-L27094	***LZTR1***	22q11.21	GCTCAATGACCT-CCTGCGGTTCGA	11.1 kb
400 + ¬	22448-L27086	***LZTR1***	22q11.21	ATTGTGCACCAG-TTCACCAAGGTC	2.8 **M**b
433 + ¬	22451-L25981	***SMARCB1***	22q11.23	TGGCGCTGAGCA-AGACCTTCGGGC	46.5 kb
319 + ¬	08294-L20837	***SMARCB1***	22q11.23	CTGAGATGGAGA-AGAAGATCCGCG	5.8 **M**b
391 « ¬	02580-L02042	*NIPSNAP1*	22q12.2	AGGCTGACAAGT-TCTGAGGATTAC	47.4 kb
***NF2* gene at 22q12.2.** Ligation sites are according to NM_000268.4.					
143	01563-L31617	Upstream	488 nt before exon 1	ACGCAGTCCCCT-GGGGCGCCACAC	0.1 kb
382	01581-L01135	Upstream	391 nt before exon 1	CCTCTAAGTGGT-TTCCCGGGTAAG	0.8 kb
		start codon	*367-369 (Exon 1)*		
244	22441-L04978	Exon 1	410-411	CAGCTCTCTCAA-GAGGAAGCAACC	32.8 kb
172	01565-L31618	Exon 2	551-552	AACCTGGTTCTT-TGGACTGCAGTA	2.3 kb
178	01566-L31619	Exon 3	682-683	CTGAGAATGCTG-AAGAGGAGCTGG	3.1 kb
197	01567-L31620	Exon 4	777-778	CCTCCTGAGGCT-TCTGTGCTCCTG	12.4 kb
208	01568-L31621	Exon 5	838-839	ACCCCAGTGTTC-ACAAGCGGGGAT	1.0 kb
270	22442-L02031	Exon 6	925-926	TGTGGGAGGAGA-GAATTACTGCTT	2.6 kb
226	18696-L29634	Exon 7	993-994	GAATATCTGAAG-ATAGCTCAGGAC	3.0 kb
254	01571-L31622	Exon 8	1094-1095	GGGGCTTCACAT-TTATGACCCTGA	3.7 kb
154	22445-L31625	Exon 9	1200-1201	CCACTGGATAAG-AAAATTGATGTC	3.4 kb
337	22443-L31616	Exon 10	1357-1356 reverse	CACCTGCTTTCT-AGCCTTCTCCTC	3.5 kb
280	15774-L17826	Exon 11	1469-1468 reverse	CGTTGGCCATTG-TTGCTTCTTCTT	1.4 kb
310	01575-L31623	Exon 12	1547-1548	CACCGAGGAGGA-GGCAAAACTTCT	1.5 kb
158	22439-L31614	Exon 13	1736-1737	GCTGAAGCAGGA-CCTGCAGGAAGC	3.4 kb
328	01577-L01149	Exon 14	1868-1869	CTTCAACCTCAT-TGGTGACAGCCT	3.2 kb
190	22440-L31615	Exon 15	1994-1995	CAATGAACTCAA-GACAGAAATCGA	2.0 kb
355 ⍟	03318-L02736	Exon 16	1.9 kb after exon 15; NM_181825.3; 2545-2546	CTGGTGTGTTTA-ACTCAAGATCAA	11.3 kb
136	22444-L31624	Exon 17	2142-2141 reverse	TAGAGCTCTTCA-AAGAAGGCCACT	0.1 kb
		stop codon	*2152-2154 (Exon 17)*		
366	01580-L29633	Exon 17	2227-2228	CAGATATCAAGA-GAGCCATCCATA	3.3 kb
217	22446-L31626	Exon 17	5568-5567 reverse	GAAGTGTTATGT-AATGCCACGGGG	30.9 kb
Telomeric flanking probes.					
409 « ¬	03317-L31857	*CABP7*	22q12.2	ACATAGAGAACA-TCATCATGACGG	3.7 **M**b
463 ¬	12460-L13461	*LARGE1*	22q12.3	AGGAATAGCTGC-ACCTTCGAACCT	7.8 **M**b
292 ¬	22449-L31628	*EP300*	22q13.2	CTACCATTAAGA-GGAAGTTAGACA	8.8 **M**b
427 ¬	22450-L31629	*ALG12*	22q13.33	TCCAGCACAATT-ATGACAATTCAG	

[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.

± 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.
¬ 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.
ⱺ This sequence is not part of the main transcript NM_000268.4 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_181825.3 (ligation site 2545-2546). 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.
+ *SMARCB1* and *LZTR1* are tumour suppressor genes associated with schwannomatosis, which is a tumour predisposition syndrome related to *NF2*-related schwannomatosis. Additionally, LOH of *NF2* in sporadic and *NF2*-related schwannomatosis-associated tumours often includes loss of *SMARCB1* and *LZTR1*. These probes also help to determine the extent of the deletion/duplication on chromosome 22.

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 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
263	08812-L24457	*DYSF*	2p13	AGTCTGAGGCTT-TCGAAGGCTTGA	02-071,630
148	14199-L23450	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG	02-108,894
472	14846-L16554	*CPOX*	3q11	CAGAATTGAAAG-TATCTTGATGTC	03-099,783
236	19652-L26684	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
416	22471-L24662	*PKD2*	4q22	CAGCTTCCCAGA-TCAGTCATGGTT	04-089,216
129	18709-L26847	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132,038
374	10718-L31856	*PKHD1*	6p12	CAGCAGGAGTAT-TACCATACAAGG	06-051,885
184	16915-L19859	*ROR2*	9q22	GGATGAAGACCA-TTACCGCCACTG	09-093,559
301	17452-L21208	*GRIN2B*	12p13	CATGCACACGGA-ATAATTACTCTG	12-014,024
346	04337-L20895	*FBN1*	15q21	GGAGCTACACCT-GTGAGTGTAATG	15-046,508
203	13121-L31675	*TGFB1I1*	16p11	CAGGAACTTAAT-GCCACTCAGTTC	16-031,393
166	16253-L31432	*RNASEH2A*	19p13	AGGACACGGACT-TTGTCGGCTGGG	19-012,779
445	05916-L14204	*HSPA13*	21q11	ATTCAGCAAGTA-TTGAAAGAAGGC	21-014,668

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

Related SALSA MLPA probemixes

P081/P082 NF1	These two probemixes contain probes for the *NF1* gene, which is associated with Neurofibromatosis type 1 (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 and schwannomatosis. The two SMARCB1 probes present in P044-C1 are also included in P258-C1.
P294 Tumour Loss	This probemix contains probes for fifteen chromosomal regions, which are frequently deleted in tumour samples. The *SMARCB1* gene is among these regions. The *SMARCB1* exon 1 probe present in P044-C1 is also included is P294-C1.

P455 LZTR1	This probemix contains probes for all exons of the *LZTR1* gene, which is associated with schwannomatosis. The two LZTR1 probes present in P044-C1 are also included in P455-A1.

References

- Abo-Dalo B et al. (2010). Large intragenic deletions of the NF2 gene: breakpoints and associated phenotypes. *Genes Chromosomes Cancer*. 49:171-5.
- Asthagiri AR et al. (2009). Neurofibromatosis type 2. *Lancet.* 373:1974-86.
- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- Evans DG et al. (2007). Mosaicism in neurofibromatosis type 2: an update of risk based on uni/bilaterality of vestibular schwannoma at presentation and sensitive mutation analysis including multiple ligation-dependent probe amplification. *J Med Genet*. 44:424-8.
- Halliday D et al. (2017). Genetic Severity Score predicts clinical phenotype in NF2. *J Med Genet*. 54:657- 664.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Kluwe L et al. (2005). Screening for large mutations of the NF2 gene. *Genes Chromosomes Cancer*. 42:384-91.
- Lassaletta L et al. (2013). NF2 genetic alterations in sporadic vestibular schwannomas: clinical implications. *Otol Neurotol*. 34:1355-61.
- Mohyuddin A et al. (2002). Molecular genetic analysis of the NF2 gene in young patients with unilateral vestibular schwannomas. *J Med Genet*. 39:315-22.
- Pathmanaban ON et al. (2017). Association of Genetic Predisposition With Solitary Schwannoma or Meningioma in Children and Young Adults. *JAMA Neurol*. 74:1123-9.
- 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.
- Smith MJ et al. (2016). The Contribution of Whole Gene Deletions and Large Rearrangements to the Mutation Spectrum in Inherited Tumor Predisposing Syndromes. *Hum Mutat*. 37:250-6.
- 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 P044 NF2

- Abo-Dalo B et al. (2010). Large intragenic deletions of the NF2 gene: breakpoints and associated phenotypes. *Genes Chromosomes Cancer.* 49:171-5.
- Castellanos E et al. (2015). Segmental neurofibromatosis type 2: discriminating two hit from four hit in a patient presenting multiple schwannomas confined to one limb. *BMC Med Genomics.* 8:2.
- Chen H et al. (2017). Differential NF2 Gene Status in Sporadic Vestibular Schwannomas and its Prognostic Impact on Tumour Growth Patterns. *Sci Rep.* 7:5470.
- Chu YW et al. (2014). A patient with mosaic neurofibromatosis type 2 presenting with early onset meningioma. *BMJ Case Rep.* 2014:bcr2014203919.
- Contini E et al. (2015). A Systematic Assessment of Accuracy in Detecting Somatic Mosaic Variants by Deep Amplicon Sequencing: Application to NF2 Gene. *PLoS One.* 10:e0129099.
- Denayer E et al. (2009). Pathogenesis of vestibular schwannoma in ring chromosome 22. *BMC Med Genet.* 10:97.
- Evans DG et al. (2007). Mosaicism in neurofibromatosis type 2: an update of risk based on uni/bilaterality of vestibular schwannoma at presentation and sensitive mutation analysis including multiple ligation dependent probe amplification. *J Med Genet.* 44:424-8.
- Evans DG et al. (2020). Incidence of mosaicism in 1055 *de novo* NF2 cases: much higher than previous estimates with high utility of next-generation sequencing. *Genet. Med.* 22:53-9.

- Forde C et al. (2021). Disease course of Neurofibromatosis Type 2; a 30-year follow-up study of 353 patients seen at a single institution. *Neuro Oncol.* 23:1113-24.
- Hadfield KD et al. (2010). Rates of loss of heterozygosity and mitotic recombination in NF2 schwannomas, sporadic vestibular schwannomas and schwannomatosis schwannomas. *Oncogene.* 29:6216-21.
- Havik AL et al. (2018). Genetic landscape of sporadic vestibular schwannoma. *J Neurosurg.* 128:911-22.
- Kehrer-Sawatzki H et al. (2018). Co-occurrence of schwannomatosis and rhabdoid tumor predisposition syndrome 1. *Mol Genet Genomic Med.* 6:627–37.
- Kehrer-Sawatzki H et al. (2018). Phenotypic and genotypic overlap between mosaic NF2 and schwannomatosis in patients with multiple non-intradermal schwannomas. *Hum Genet.* 137:543-52.
- Kluwe L et al. (2005). Screening for large mutations of the NF2 gene. *Genes Chromosomes Cancer.* 42:384-91.
- Louvrier C et al. (2018). Targeted next-generation sequencing for differential diagnosis of neurofibromatosis type 2, schwannomatosis, and meningiomatosis. *Neuro Oncol.* 20:917-29.
- Martinez-Glez V et al. (2007). Multiplex ligation-dependent probe amplification (MLPA) screening in meningioma. *Cancer Genet Cytogenet.* 173:170-2.
- Martinez-Glez V et al. (2009). Meningiomas and schwannomas: molecular subgroup classification found by expression arrays. *Int J Oncol.* 34:493-504.
- Pasmant E et al. (2015). Neurofibromatosis type 2 French cohort analysis using a comprehensive NF2 molecular diagnostic strategy. *Neurochirurgie.* 64:335-41.
- Rosset C et al. (2018). Clinical and molecular characterization of neurofibromatosis in southern Brazil. *Expert Rev Mol Diagn.* 18:577-86.
- Rostasy K et al. (2013). Modification of risk for cancer as a coincidental finding in DNA array investigation. *Clin Genet.* 83:284-7.
- Shen Y et al. (2009). Genomic profiling distinguishes familial multiple and sporadic multiple meningiomas. *BMC Med Genomics.* 2:42.
- Smith MJ et al. (2016). The Contribution of Whole Gene Deletions and Large Rearrangements to the Mutation Spectrum in Inherited Tumor Predisposing Syndromes. *Hum Mutat.* 37:250-6.
- Tritto V et al. (2022). Characterization of 22q12 Microdeletions Causing Position Effect in Rare NF2 Patients with Complex Phenotypes. *Int J Mol Sci.* 23:10017.
- Zirn B et al. (2012). Ring chromosome 22 and neurofibromatosis type II: proof of two-hit model for the loss of the NF2 gene in the development of meningioma. *Clin Genet.* 81:82-7.

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 C1-04 – 15 January 2024 (04P) - Warning removed from Table 1 and 2 for SNP rs1800538 influence on probe signal of probe 01581-L01135 at 382 nt. - Disease nomenclature updated to *NF2*-related schwannomatosis (previously NF2) throughout this product description, including the Intended Purpose. - Removed information about transcript variants 1 and 2 being RefSeqGen standard/predominant in 'Transcript variants' section on page 2. - Footnote "¬ Flanking probe." added for LZTR1, EP300 and SMARCB1 probes in Tables 1 and 2.

- Table 3 with reference probe locations added.
- One selected publication added.
- Various minor textual changes, including layout modifications in Table 1 and 2

Version C1-03 – 21 March 2022 (04P)
- Warning added to Table 1 and 2 for salt sensitivity of 409 nt probe.
- Minor textual changes.

Version C1-02 – 10 May 2021 (04P)
- Product description adapted to a new template.
- UK has been added to the list of countries in Europe that accept the CE mark.
- Ligation sites of the probes targeting the *NF2* gene updated according to new version of the NM reference sequence.
- Two selected publications added.
- Minor textual and layout corrections.

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).

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IVD	EUROPE* CE
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.