Product Description
SALSA® MLPA® Probemix P472-A1 SUFU

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 7.

Catalogue numbers:
- **P472-025R:** SALSA MLPA Probemix P472 SUFU, 25 reactions.
- **P472-050R:** SALSA MLPA Probemix P472 SUFU, 50 reactions.
- **P472-100R:** SALSA MLPA Probemix P472 SUFU, 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.

General information
The SALSA MLPA Probemix P472 SUFU is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SUFU* gene, which is associated with Gorlin syndrome and predisposition to several cancer types.

The *SUFU* gene (OMIM 607035) encodes a component of the Sonic hedgehog (SHH)/Patched (PTCH) signalling pathway. Mutations in the *SUFU* gene are expected to result in the same clinical phenotype as mutations in the better known *PTCH1* gene (OMIM 601309). Screening for the *SUFU* gene is therefore suggested when the *PTCH1* gene is wildtype in patients with clinical basal cell nevus (Gorlin) syndrome (OMIM#109400). Germline mutations in the *SUFU* gene are suggested to predispose to infant desmoplastic/ nodular medulloblastomas, basal cell carcinomas and meningiomas. This *SUFU* susceptibility gene shows autosomal dominant inheritance with an incomplete penetrance. In addition to point mutations, both whole *SUFU* gene duplications and partial *SUFU* gene deletions have been described (Brugieres et al. 2012; Smith et al. 2014; Kenawy et al. 2019).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1151/

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering
The *SUFU* exon numbering used in this P472-A1 SUFU product description is the exon numbering from the LRG_521 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 P472-A1 SUFU contains 30 MLPA probes with amplification products between 121 and 317 nucleotides (nt). This includes 15 probes for the *SUFU* gene and two probes flanking the *SUFU* gene. In addition, 13 reference probes are included that target relatively copy number stable regions in meningiomas and medulloblastomas. Complete 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, which includes DNA derived from paraffin-embedded tissues, 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. Information on the use of formalin-fixed paraffin-embedded 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. For germline analysis reference samples should be derived from different unrelated individuals who are from families without a history of Gorlin syndrome, and for tumour analysis from healthy individuals without a history of cancer. 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. Sample ID numbers NA00959, NA08386 and NA20125 from

the Coriell Institute, and ACC-203, ACC-259 and ACC-569 from the Leibniz Institute DSMZ have been tested with this P472-A1 probemix at MRC Holland and can be used as a positive control samples to detect *SUFU* deletion/duplication. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Copy number alteration position*	Altered target genes in P472-A1	Expected copy number alteration
NA00959	Coriell Institute	10q24.32	*ACTR1A*, ***SUFU***, *TRIM8*	Heterozygous duplication
NA08386				
NA20125				
ACC-203	Leibniz Institute DSMZ	10q24.32	*ACTR1A*, ***SUFU***, *TRIM8*	Heterozygous deletion
ACC-259				
ACC-569				

* Indicated hg18 chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P472-A1 SUFU probemix.

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 standard deviation of each individual probe over all the reference samples should be ≤0.10, and for germline analysis 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.

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 or 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.
- 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.

Limitations of the procedure

- In most populations, most genetic alterations in *SUFU* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P472 SUFU.
- 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 solid tumours with more chaotic karyotypes.

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.

COSMIC mutation database

http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the Catalogue of Somatic Mutations in Cancer (COSMIC) 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 during germline analysis, false positive results due to SNVs and unusual results (e.g., a duplication of *SUFU* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P472-A1 SUFU

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SUFU*
64-105	Control fragments – see table in probemix content section for more information		
121	Reference probe S0864-L27364	21q22	
129	Reference probe 19616-L26684	4p13	
135	Reference probe 19551-L26642	2p13	
140	**SUFU probe** 21047-L29276		Exon 4
146	**SUFU probe** 21048-L29277		Exon 1
151	**SUFU probe** 21049-L29278		Exon 11
157	Reference probe 07118-L29026	12p13	
162	**SUFU probe** 21050-L29279		Exon 9
169	**SUFU probe** 21051-L29280		Exon 12
178	Reference probe 04857-L28909	5p13	
185	**SUFU probe** 21053-L29282		Exon 7
190	**SUFU probe** 21054-L29283		Exon 12
196	**SUFU probe** 21055-L29541		Exon 5
202	Reference probe 18721-L24087	2q36	
210	**SUFU probe** 21056-L29285		Exon 3
218 Ø	**SUFU probe** 21057-L29286		Intron 10
224	**SUFU probe** 21279-L29642		Exon 6
229	Reference probe 20525-L29005	1q31	Exon 3
239	**SUFU probe** 21060-L29289		Exon 3
246	**SUFU probe** 21061-L29535		Exon 10
250	Reference probe 06712-L29006	15q24	
257	**SUFU probe** 21062-L29291		Exon 8
265	Reference probe 16433-L29008	18q21	
271	**SUFU probe** 21064-L29293		Exon 2
277	Reference probe 13796-L24815	3q25	
286	Reference probe 18858-L24382	3p14	
292 ¬	ACTR1A probe 21065-L29294		10q24.32
302	Reference probe 06548-L28789	5q13	
310 ¬	TRIM8 probe 21066-L29542		10q24.32
317	Reference probe 11898-L24065	6p12	

[a] See section Exon numbering on page 1 for more information.

¬ 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 Intron probe. 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. Single probe aberration(s) must be confirmed by another method.

Table 2. *SUFU* 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
SUFU flanking probe – upstream.					
292 ¬	21065-L29294	*ACTR1A*	10q24.32	CCTCACCTCCTC-TGAGAAAGTCTG	16.1 kb
SUFU gene at 10q24.32. Ligation site and start/stop codon locations are according to NM_016169.4 (except intron 10).					
		start codon	*182-184 (Exon 1)*		
146	21048-L29277	Exon 1	320-321	GCCTTTACCCTG-ACCAGCCGAACC	4.9 kb
271	21064-L29293	Exon 2	384-385	CCCAGACCCCTT-GGACTATGTTAG	40.8 kb
239	21060-L29289	Exon 3	535-536	GGTTTTGGCTTT-GAGTTGACCTTT	0.1 kb
210	21056-L29285	Exon 3	605-606	CAGAGTTAATGC-AGGGCTTGGCAC	42.6 kb
140	21047-L29276	Exon 4	754-753 reverse	ACTACCCCAAAG-GGTGTCTGCACG	1.0 kb
196	21055-L29541	Exon 5	814-815	CTACACTCAGCC-CAGCAGTGGAAC	0.4 kb
224	21279-L29642	Exon 6	918-919	GACCATATTTGA-GATCGATCCACA	3.1 kb
185	21053-L29282	Exon 7	973-974	ACAGATGGCTCC-AACCTGAGTGGT	2.3 kb
257	21062-L29291	Exon 8	1148-1147 reverse	TGGTGGAAGGAC-AGGTTTGCTGTT	15.9 kb
162	21050-L29279	Exon 9	1295-1296	GCGTACATCTGA-AATTCAACCAGG	2.0 kb
246	21061-L29535	Exon 10	1385-1386	AAAGTATCACAG-GTGACATGGCCA	1.8 kb
218 Ø	21057-L29286	Intron 10	NM_001178133.2; 1560-1559 reverse	GAGCTGCTGAAA-ATTGGAGATGCT	8.1 kb
151	21049-L29278	Exon 11	1538-1537 reverse	TACTTCCTCTGG-AGAAGTCAAATC	2.9 kb
190	21054-L29283	Exon 12	1611-1612	CCTGCCTGACGT-GGTGTTCGACAG	2.3 kb
		stop codon	*1634-1636 (Exon 12)*		
169	21051-L29280	Exon 12	3950-3949 reverse	GAGGCCTGGTGA-GAAATGTGTGAT	22.2 kb
SUFU flanking probe – downstream.					
310 ¬	21066-L29542	*TRIM8*	10q24.32	CATTGAGGACCA-GCTGTACAAACT	-

[a] See section Exon numbering on page 1 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.

¬ 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 Intron probe. 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. 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
229	20525-L29005	*CDC73*	1q31	TGGTTAGAAGAC-CTGATCGAAAAG	01-191,366
135	19551-L26642	*DYSF*	2p13	CCATTGCCAAGA-AGGTCAGTGTCC	02-071,750
202	18721-L24087	*COL4A3*	2q36	GCAACTACTATT-CAAATTCCTACA	02-227,884
286	18858-L24382	*FLNB*	3p14	AGAGAAGTGATT-ATGTATTTCTCA	03-058,059
277	13796-L24815	*KCNAB1*	3q25	CTTTTCCAGAGA-GAGAAAGTGGAG	03-157,716
129	19616-L26684	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
178	04857-L28909	*NIPBL*	5p13	CTGCAATGTTGC-AAAAATCCTAGA	05-037,080
302	06548-L28789	*MCCC2*	5q13	TCCAGTTATGCT-GCCAAAGAAATA	05-070,978
317	11898-L24065	*PKHD1*	6p12	GTGTTTCCAGAA-ACTGGGAGCCTT	06-052,039
157	07118-L29026	*FGF23*	12p13	CAGATCAGAGGA-TGCTGGCTTTGT	12-004,352
250	06712-L29006	*HEXA*	15q24	GAATGTGTTGGT-TGTCTCTGTAGT	15-070,436
265	16433-L29008	*MYO5B*	18q21	GCTCCAGCAGCA-GTTCAACTCGGT	18-045,743
121	S0864-L27364	*KCNJ6*	21q22	AGCTCCTACATC-ACCAGTGAGATC	21-037,920

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

Related SALSA MLPA probemixes

- P044 NF2: Contains probes for all exons of the *NF2* gene, involved in familial meningioma.
- P067 PTCH1: Contains probes for 23 out of 25 exons of the *PTCH1* gene, involved in basal cell nevus (Gorlin) syndrome.
- P225 PTEN: Contains at least two probes for all exons of the *PTEN* gene, involved in familial meningioma.

References

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P472 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-01 – 21 December 2020 (04P) - Product description rewritten and adapted to a new template. - Exon numbering of the 21057-L29286 probe has been changed to Intron 10. - Ligation sites of the probes targeting the *SUFU* gene updated according to new version of the NM_ reference sequence. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). *Version 01 – 17 March 2017 (T08)* - Not applicable, new document.

More information: www.mrcholland.com; www.mrcholland.eu	
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