Product Description
SALSA® MLPA® Probemix P461-B1 STRC-CATSPER2-OTOA

To be used with the MLPA General Protocol.

Version B1
As compared to version A1, new target probes, amongst others probes for STRCP1, have been added, and several target and flanking probes have been removed. Most reference probes have been replaced. For complete product history see page 10.

Catalogue numbers:
- **P461-025R:** SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA, 25 reactions.
- **P461-050R:** SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA, 50 reactions.
- **P461-100R:** SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA, 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 P461 STRC-CATSPER2-OTOA is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *STRC*, *CATSPER2* and *OTOA* genes, as well as gene conversions between *STRC* and its pseudogene *STRCP1*, in genomic DNA isolated from human peripheral whole blood specimens. P461 STRC-CATSPER2-OTOA is intended to confirm a potential cause for and clinical diagnosis of Deafness-infertility syndrome (*STRC* and *CATSPER2*), Autosomal recessive deafness 16 (*STRC*) and Autosomal recessive deafness 22 (*OTOA*) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P461 STRC-CATSPER2-OTOA should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Numerous defects in the *STRC* and *OTOA* genes 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, parental evaluation, 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, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[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

Deafness-infertility syndrome (DIS; OMIM # 611102) is characterized by early-onset deafness in males and females and infertility exclusively in males. The hearing loss in DIS patients is non-progressive and the vestibular function is normal. Furthermore, the degree of hearing loss is moderate to severe. DIS is inherited in an autosomal recessive manner, as a contiguous gene deletion syndrome resulting from a homozygous deletion of the *CATSPER2-STRC* genes.

The *STRC* gene has also been associated to autosomal recessive deafness 16 (DFNB16; OMIM # 603720), a nonsyndromic hearing loss, with prelingual onset. The auditory phenotype in DFNB16 is similar to the auditory phenotype in DIS, where the hearing loss is moderate to severe. DFNB16 is caused by homozygous or compound heterozygous mutations, small indels, multi-exon or complete *STRC* deletions, as well as gene conversions between the *STRC* gene and its pseudogene (*STRCP1*) which are located less than 100 kb from each other (Moteki *et al*. 2016, Vona *et al*. 2015).

CNVs are a common cause of nonsyndromic hearing loss, *STRC* CNVs are the most common followed by CNVs in *OTOA and GJB6* (Shearer *et al*. 2014). Homozygous or compound heterozygous missense, splice site mutations or complete *OTOA* deletions cause autosomal recessive deafness 22 (DFNB22; OMIM # 607039). DFNB22 has a prelingual onset, and the degree of hearing loss has been reported as moderate to severe and severe to profound.

More information is available at:
DIS: https://www.omim.org/entry/611102; https://www.ncbi.nlm.nih.gov/books/NBK22925/;
Hereditary hearing loss and deafness: https://www.ncbi.nlm.nih.gov/books/NBK1434/;
DFNB16: https://www.omim.org/entry/603720;
DFNB22: https://www.omim.org/entry/607039.

Gene structure

The *STRC* gene spans ~19 kilobases (kb) on chromosome 15q15.3 and contains 29 exons. No LRG is available for *STRC,* the GenBank is NG_011636.1.
The *CATSPER2* gene spans ~18 kb on chromosome 15q15.3 and contains 14 exons. No LRG is available for *CATSPER2,* the GenBank is NG_009283.1.
The *OTOA* gene spans ~97 kb on chromosome 16p12.2 and contains 33 exons. No LRG is available for *OTOA,* the GenBank is NG_012973.2.

Transcript variants

For *STRC,* one transcript variant has been described encoding the full length protein (NM_153700.2; 5515 nt; coding sequence 79-5406; https://www.ncbi.nlm.nih.gov/gene/161497). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 29.
For *CATSPER2,* multiple variants have been described. Transcript variant 2 is the most predominant and encodes isoform 2 (NM_172095.4; 4009 nt; coding sequence 219-1811; https://www.ncbi.nlm.nih.gov/gene/117155). The ATG translation start site is located in exon 2 and the stop codon is located in exon 13.
For *OTOA,* multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform 1 (NM_144672.4; 3878 nt; coding sequence 270-3689; https://www.ncbi.nlm.nih.gov/gene/146183). The ATG translation start site is located in exon 2 and the stop codon is located in exon 29.

Exon numbering

The exon numbering used in this P461-B1 STRC-CATSPER2-OTOA product description is the exon numbering from the RefSeq transcript NM_153700.2 for *STRC,* RefSeq transcript NM_172095.4 for *CATSPER2* and RefSeq transcript NM_144672.4 for *OTOA*. As changes to the databases can occur after release of this product description, the NM_ sequences and exon numbering may not be up-to-date.

Probemix content

The SALSA MLPA Probemix P461-B1 STRC-CATSPER2-OTOA contains 45 MLPA probes with amplification products between 124 and 490 nt. This includes seven probes for the *STRC* gene, five probes for the *CATSPER2* gene and ten probes for the *OTOA* gene. Multiple flanking probes are included for the aforementioned genes in the 15q15.3 and 16q12.2 regions, indicating the extent of possible CNVs. Furthermore, four STRCP1 probes are present, for which the results should be interpreted together with the STRC probes for the respective exon as rearrangements or gene conversions can occur (see Table 1 Probe pairs). In addition, 11 reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

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).

MLPA technique validation

Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens

Extracted DNA from peripheral blood, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples

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 DIS or hereditary hearing loss. 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 NA03184, NA08039, NA13031, NA20511, NA20317 from the Coriell Institute have been tested with this P461-B1 probemix at MRC Holland and can be used as a positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Sample ID Coriell	Altered target genes in P461-B1	Expected copy number alteration
NA03184	*STRC; CATSPER2*	Heterozygous duplication of *STRC, CATSPER2, STRCP1* including the flanking genes
NA08039	*OTOA*	Heterozygous duplication of *OTOA* including the flanking genes
NA13031	*OTOA*	Heterozygous deletion of *OTOA* including the flanking gene located telomeric
NA20511	*STRC; CATSPER2; CKMT1B*	Heterozygous deletion of *STRC*, *CATSPER2* and *CKMT1B*
NA20317	*STRC*; *STRCP1; CKMT1B; CKMT1A*	Heterozygous duplication of *STRC*; heterozygous deletion of *STRCP1;* Heterozygous deletion of *CKMT1B;* heterozygous duplication of *CKMT1A*

Performance characteristics

The expected number of homozygous deletions of whole *STRC-CATSPER2* genes that can be detected with this MLPA probemix is 100% of all mutations in DIS patients. The expected number of *STRC* or *OTOA* deletions/duplications that can be detected with this MLPA probemix is ~68%, and ~57%, of all mutations in DFNB16 and DFNB22 patients, respectively (based on pathogenic mutations reported in https://www.ncbi.nlm.nih.gov/clinvar, which might not be fully representative). The analytical sensitivity and specificity (based on an analytical performance study and on a 2005-2021 literature review) for the detection of deletions and duplication in *STRC, CATSPER2* and *OTOA* genes is very high and can be considered >99%.

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 STRC, CATSPER2 and OTOA specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 4 (heterozygous triplication/homozygous duplication) or 0 (homozygous deletion). The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders

as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *CATSPER2* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- 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.

P461 specific notes

- The *STRC, CATSPER2* and *OTOA* genes have highly homologous pseudogenes. In rare cases, apparent duplications might therefore be due to sequence changes in the homologous genes.
- Gene conversion between *STRC* and *STRCP1* can occur at several sites. Although, up to date the only known clinical significant gene conversion is a nonsense mutation in exon 20, leading to a stop codon (Vona et al. 2015).
- As rearrangements or gene conversions can occur, probe pairs were designed for a number of *STRC* and *STRCP1* exons (19, 20, 23 and 28; see Table 1 and 2a for probe pairs). To facilitate interpretation of results, *STRCP1* exon numbering is adjusted to the corresponding exons of *STRC*.

Limitations of the procedure

- In most populations, genetic defects in the *STRC* and *OTOA* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

Mutation databases

https://databases.lovd.nl/shared/genes/STRC;
https://databases.lovd.nl/shared/genes/CATSPER2;
https://databases.lovd.nl/shared/genes/OTOA.

We strongly encourage users to deposit positive results in the LOVD Database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P461-B1 STRC-CATSPER2-OTOA

Length (nt)	Probe pairs[a]	SALSA MLPA probe	Chromosomal position (hg18)[b]		
			Reference	15q15.3	16p12.2
64-105		Control fragments – see table in probemix content section for more information			
124 *		Reference probe 19616-L26275	4p		
136 ¬		UQCRC2 probe 20583-L28241			Centromeric
143 ¥		**OTOA probe** 22686-L28243			**Exon 7**
149 * ¬ ‡	**A**	CKMT1A probe 22682-L31933		Telomeric	
155 ¥		**OTOA probe** 20584-L32088			**Exon 2**
160 *		Reference probe 09267-L09527	10q		
166 ±		**STRC probe** 20146-L27402		**Exon 24**	
177 *		Reference probe 04359-L03779	7p		
187 * ‡	**B**	**STRC probe** 22755-L32078		**Exon 28**	
190 ¥ ¬	**A**	CKMT1B probe 20147-L31814		Centromeric	
202 *		**OTOA probe** 22597-L31809			**Exon 18**
208 * ‡ ±	**C**	**STRCP1 probe** 22753-L32338		**Exon 19**	
214 *		Reference probe 10698-L11280	6p		
220 ¬ ±		PPIP5K1 probe 20151-L27407		Centromeric	
226 *		**STRC probe** 22602-L31815		**Exon 25**	
232		**OTOA probe** 20586-L28244			**Exon 17**
244 ¥ ±		**STRC probe** 20155-L32077		**Exon 19**	
250 *		Reference probe 15065-L16823	15q		
256 * ‡	**D**	**STRCP1 probe** 22607-L31820		**Exon 23**	
263 ¬		PDIA3 probe 20157-L28761		Telomeric	
267 *		Reference probe 19015-L32525	21q		
274 * «		**CATSPER2 probe** 22593-L32079		**Exon 1**	
290 ¬		METTL9 probe 20588-L28718			Telomeric
297 «		**CATSPER2 probe** 20160-L28719		**Exon 7**	
303 «		**CATSPER2 probe** 20236-L28905		**Exon 2**	
311 * ‡	**C**	**STRC probe** 22754-L32091		**Exon 19**	
319 *		Reference probe 06580-L30872	2q		
328 ¥ ¬		UQCRC2 probe 23045-L28255			Centromeric
337		**OTOA probe** 20590-L28248			**Exon 16**
346 *		**OTOA probe** 22595-L31807			**Exon 8**
352 *		Reference probe 13400-L20982	6q		
364 * ‡	**E**	**STRCP1 probe** 22608-L31821		**Exon 20**	
373 *		**OTOA probe** 22596-L31808			**Exon 12**
380 *	**D**	**STRC probe** 22603-L31816		**Exon 23**	
388 * ‡	**B**	**STRCP1 probe** 22684-L31935		**Exon 28**	
394		Reference probe 03178-L18979	12p		
407 ¥		**OTOA probe** 23046-L28256			**Exon 5**
418		**OTOA probe** 20599-L28257			**Exon 11**
433 ¬		METTL9 probe 20600-L28841			Telomeric
444 * «		**CATSPER2 probe** 22606-L31819		**Exon 1**	
454 *		**OTOA probe** 22598-L31810			**Exon 20**
463 *		Reference probe 10108-L10532	8q		
472 * ‡	**E**	**STRC probe** 22604-L31817		**Exon 20**	
483 * «		**CATSPER2 probe** 22592-L31804		**Exon 4**	
490 *		Reference probe 20096-L27538	4p		

[a] As rearrangements or gene conversions can occur, probe pairs were designed for a number of *STRC* and *STRCP1* exons (19, 20, 23 and 28; see column of probe pairs). To facilitate interpretation of results, *STRCP1* exon numbering is adjusted to the corresponding exons of *STRC*.

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

* New in version B1.

¥ Changed in version B1. Minor alteration, no change in sequence detected.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

¬ 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.

‡ This probe is sensitive to sample DNA depurination. Use of depurinated DNA will lead to a lower signal of this probe. Depurination can occur in acidic conditions, e.g. due to insufficient buffering capacity during sample DNA denaturation. When this occurs only in reference samples, it will result in an increased ratio in the test samples.

± SNP rs141809944 can influence the 166 nt probe signal. SNP rs188647272 can influence the 208 nt probe signal. SNP rs551423552 can influence the 220 nt probe signal. SNP rs2614832 can influence the 244 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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. P461-B1 probes arranged according to chromosomal location

Table 2a. 15q15.3

Length (nt)	Probe pairs[a]	SALSA MLPA probe	Gene exon[b]	Distance to next probe
220 ¬ ± #		20151-L27407	*PPIP5K1*	40.1 kb
190 ¬	A	20147-L31814	*CKMT1B*; Exon 10	0.9 kb
			STRC	
187 ‡	B	22755-L32078	Exon 28	0.8 kb
226		22602-L31815	Exon 25	0.6 kb
166 ± #		20146-L27402	Exon 24	1.8 kb
380	D	22603-L31816	Exon 23	1.5 kb
472 ‡	E	22604-L31817	Exon 20	0.4 kb
244 ± #		20155-L32077	Exon 19	0.2 kb
311 ‡	C	22754-L32091	Exon 19	33.6 kb
			CATSPER2	
297 « #		20160-L28719	Exon 7	8.0 kb
483 « #		22592-L31804	Exon 4	0.8 kb
303 « #		20236-L28905	Exon 2	0.8 kb
444 « #		22606-L31819	Exon 1	0.2 kb
274 «		22593-L32079	Exon 1	50.1 kb
149 ¬ ‡	A	22682-L31933	*CKMT1A*; Exon 10	0.9 kb
			STRCP1	
388 ‡ ◎	B	22684-L31935	Exon 28	2.9 kb
256 ◎	D	22607-L31820	Exon 23	1.4 kb
364 ‡ ◎	E	22608-L31821	Exon 20	0.7 kb
208 ‡ ± ◎	C	22753-L32338	Exon 19	41.8 kb
263 ¬ #		20157-L28761	*PDIA3*	

Table 2b. 16p12.2

Length (nt)	SALSA MLPA probe	Gene exon[b]	Ligation site	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
290 ¬	20588-L28718	*METTL9* Exon 2		AGAAATCGGGCT-GGCTATTTATCC	12.3 kb
433 ¬	20600-L28841	*METTL9* Exon 4		AGGACAAGGGCA-AGGATGACCCTG	53.5 kb
		OTOA	**NM_144672.4**		
		start codon	*70-72 (Exon 2)*		
155	20584-L32088	Exon 2	351-352	CAGTGCCAAATT-CCAGGCAGGGTA	0.6 kb
407	23046-L28256	Exon 5	1 nt before exon 5 reverse	ATCCAGCAGTGC-TACAATGGGAGT	6.1 kb
143	22686-L28243	Exon 7	625-624 reverse	TCATGGCAGTGC-GGAACTGCTGGG	2.1 kb
346	22595-L31807	Exon 8	701-702	GACTTAGGAGAG-ATTCGAGAACGA	13.6 kb
418	20599-L28257	Exon 11	1216-1217	CAGCTCCTCCAA-CTTTAACATGAG	4.3 kb
373	22596-L31808	Exon 12	1338-1339	TGATCAAGTGCA-GCCACCTGAGGG	13.9 kb
337	20590-L28248	Exon 16	1927-1928	GCTTCTGTTAAA-GACCACCAGAAG	0.3 kb
232	20586-L28244	Exon 17	2013-2012 reverse	CAGAAAGAAGTC-AGTGCTCATGGC	3.5 kb
202	22597-L31809	Exon 18	2132-2133	ATGCCACCTTTC-CTCTTGGCTGCA	5.3 kb
454	22598-L31810	Exon 20	2298-2299	ACGACTCCATTG-CTGATGAGTACA	229.3 kb
		stop codon	*3687-3689 (Exon 29)*		
136 ¬	20583-L28241	*UQCRC2* Exon 4		TTTCCAAACACT-TGAGGAAGTGAA	16.4 kb
328 ¬	23045-L28255	*UQCRC2* Exon 11		GTATAAATCCCA-AAGAGTCCAGAA	

[a] As rearrangements or gene conversions can occur, probe pairs were designed for a number of *STRC* and *STRCP1* exons (19, 20, 23 and 28; see column of probe pairs). To facilitate interpretation of results, *STRCP1* exon numbering is adjusted to the corresponding exons of *STRC*.

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

[c] Only partial probe sequences are shown. Complete probe sequences of the 16p12.2 region and reference probes 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.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

‡ This probe is sensitive to sample DNA depurination. Use of depurinated DNA will lead to a lower signal of this probe. Depurination can occur in acidic conditions, e.g. due to insufficient buffering capacity during sample DNA denaturation. When this occurs only in reference samples, it will result in an increased ratio in the test samples.

± SNP rs141809944 can influence the 166 nt probe signal. SNP rs188647272 can influence the 208 nt probe signal. SNP rs551423552 can influence the 220 nt probe signal. SNP rs2614832 can influence the 244 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

ⵙ The clinical significance of aberrations of STRCP1 probes is not known. Probes for *STRCP1* are included as an aid to differentiate between *STRC* CNVs and possible gene conversions.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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

Related SALSA MLPA probemixes

P153 EYA1	Contains probes for the *EYA1* gene involved in bronchi-oto-renal dysplasia, causing profound deafness in children.
P163 GJB-WFS1-POU3FA	Contains probes for the *GJB2*, *GJB6*, *GJB3*, *WFS1* and *POU3F4* genes involved in prelingual deafness.
P280 SLC26A4	Contains probes for *SLC26A4* gene involved in DFNB4 and Pendred syndrome.

References

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Selected publications using SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA

- Cabanillas R et al. (2018). Comprehensive genomic diagnosis of non-syndromic and syndromic hereditary hearing loss in Spanish patients. *BMC Med Genomics*. 11:58.
- Kim BJ et al. (2020). Significant Mendelian genetic contribution to pediatric mild-to-moderate hearing loss and its comprehensive diagnostic approach. *Genet Med.* 22:1119-1128.
- Laurent S et al. (2021). Molecular characterization of pathogenic OTOA gene conversions in hearing loss patients. *Human Mutation*. 42:373-377.
- Markova SP et al. (2018). STRC Gene Mutations, Mainly Large Deletions, are a Very Important Cause of Early-Onset Hereditary Hearing Loss in the Czech Population. *Genet Test Mol Biomarkers*. 22:127-34.
- Morgan A et al. (2020). Lights and Shadows in the Genetics of Syndromic and Non-Syndromic Hearing Loss in the Italian Population. *Genes (Basel).* 11.
- Plevova P et al. (2017). STRC Deletion is a Frequent Cause of Slight to Moderate Congenital Hearing Impairment in the Czech Republic. *Otol Neurotol*. 38:e393-e400.

P461 product history	
Version	*Modification*
B1	New target probes, amongst others probes for STRCP1, have been added, and several target and flanking probes have been removed. Most reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version B1-01 – 01 December 2021 (04P) - Product description rewritten and adapted to a new template. - P461-B1 is now CE marked. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Product name changed from SALSA MLPA Probemix P461 DIS to SALSA MLPA Probemix P461 STRC-CATSPER2-OTOA Version A1-01 - 11 February 2019 (01P) - Product description restructured and adapted to a new template.

- Exon numbering of the *OTOA* gene has been changed in Table 1 and 2b.
- Ligation sites of the probes targeting the *OTOA* and the *CATSPER2* genes updated according to new version of the NM_reference sequence.
- Warning added to Table 1 and Table 2a for salt sensitive probe, 297 nt probe 20160-L28719.
- Warning added to Table 1 and Table 2a for more variable probe, 160 nt probe 20145-L27401.
- Warning added to Table 1 and Table 2a for flanking probes, 160 nt probe 20145-L27401 and 184 nt probe 20147-L27403.
- Warnings added for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene to Table 2.

Version 02 – 15 June 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes on page 1.

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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.