Product Description SALSA® MLPA® probemix P361-A3/P362-A3 USH2A

To be used with the MLPA General Protocol.

P361 Version-A3. As compared to version A2, three reference probes have been replaced. For complete product history see page 9.
P362 Version-A3. As compared to version A2, two reference probes have been replaced. For complete product history see page 9.

Catalogue numbers:
- **P361-025R:** SALSA® MLPA® probemix P361 USH2A mix 1, 25 reactions.
- **P361-050R:** SALSA® MLPA® probemix P361 USH2A mix 1, 50 reactions.
- **P362-025R:** SALSA® MLPA® probemix P362 USH2A mix 2, 25 reactions.
- **P362-050R:** SALSA® MLPA® probemix P362 USH2A mix 2, 50 reactions.

To be used in combination with a SALSA® MLPA® reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General Information: The SALSA MLPA Probemix P361 and P362 USH2A is a **research use only (RUO)** assay for the detection of deletions or duplications in the *USH2A* gene.

Usher syndrome is a clinically and genetically heterogeneous autosomal recessive disorder characterized by sensorineural hearing deficiencies at birth and later development of progressive retinitis pigmentosa (RP). It is the most frequent cause of combined deafness and blindness in adults and affects 3 to 6% of children born with hearing impairment. In brief, patients with Usher syndrome type II have mild hearing impairment with normal vestibular responses. Type II is the most common form of three Usher syndromes. Mutations within the *USH2A* gene have been associated with Usher syndrome type IIa and retinitis pigmentosa.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1341

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 exon numbering used in this P361-A3/P362-A3 USH2A product description is the exon numbering from the RefSeq transcript NM_206933.2 for *USH2A.* The exon numbering and NM sequence used have been retrieved in July 2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA probemixes P361-A3 USH2A mix 1 and P362-A3 USH2A mix 2 both contain 45 probes with amplification products between 130 nt and 454 nt, and between 130 nt and 477 nt respectively. The *USH2A* gene (72 exons) spans ~800 kb of genomic DNA, and is located on chromosome 1q41, ~216 Mb from the p-telomere. The P361-A3 and P362-A3 probemixes together contain one probe for

each exon of the *USH2A* gene. The P361-A3 probemix contains probes for the odd exon numbers of *USH2A* and the P362-A3 probemix contains probes for the even exon numbers of *USH2A.* In both probemixes, nine reference probes are included, that detect different autosomal chromosomal locations.

These probemixes contain nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity Fragments (Q-fragments). two DNA Denaturation Fragments (D-fragments), one benchmark fragment, one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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 unrelated individuals who are from families without a history of sensorineural hearing disease. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

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

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *USH2A* 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *USH2A* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P361-A3 and P362-A3.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

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.

NCL gene variant database:
https://databases.lovd.nl/shared/genes/USH2A. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *USH2A* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1a. SALSA MLPA Probemix P361-A3 USH2A mix 1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*USH2A*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L13645	5q31	
136	**USH2A** probe 11385-L12111		**Exon 5**
142	**USH2A** probe 11386-L12112		**Exon 57**
148	**USH2A** probe 11387-L12113		**Exon 31**
154	**USH2A** probe 11388-L12114		**Exon 25**
160	Reference probe 02493-L20011	17q11	
166	**USH2A** probe 11389-L12115		**Exon 9**
173	**USH2A** probe 11390-L20627		**Exon 71**
178	**USH2A** probe 11391-L12117		**Exon 13**
184	**USH2A** probe 11392-L12118		**Exon 67**
190	Reference probe 09561-L19082	20p13	
196	**USH2A** probe 11393-L12119		**Exon 23**
202	**USH2A** probe 11394-L12120		**Exon 29**
208	**USH2A** probe 16841-L19625		**Exon 43**
214	**USH2A** probe 11396-L12122		**Exon 53**
220 *	Reference probe 21710-L30368	15q21	
226	**USH2A** probe 11397-L12123		**Exon 1**
232	**USH2A** probe 11398-L12124		**Exon 51**
238	**USH2A** probe 11399-L12125		**Exon 27**
244	**USH2A** probe 11400-L12126		**Exon 3**
250 *	Reference probe 17332-L24586	3p21	
256	**USH2A** probe 11401-L12127		**Exon 37**
261	**USH2A** probe 11402-L12128		**Exon 19**
268	**USH2A** probe 16840-L29088		**Exon 41**
274	**USH2A** probe 11403-L12129		**Exon 61**
283	**USH2A** probe 11404-L12130		**Exon 11**
292 *	Reference probe 17041-L20104	10p11	
301	**USH2A** probe 11405-L12131		**Exon 39**
311	**USH2A** probe 11406-L20628		**Exon 7**
319	**USH2A** probe 11407-L12133		**Exon 21**
328	**USH2A** probe 11408-L12134		**Exon 55**
342	**USH2A** probe 20574-L20015		**Exon 47**
348	Reference probe 10713-L29089	6p12	
355	**USH2A** probe 11410-L12136		**Exon 69**
366	**USH2A** probe 11411-L20629		**Exon 15**
374	**USH2A** probe 11412-L12138		**Exon 65**
382	**USH2A** probe 11409-L19614		**Exon 17**
391	**USH2A** probe 11413-L12139		**Exon 33**
400	**USH2A** probe 11414-L12140		**Exon 63**
409	**USH2A** probe 11415-L12141		**Exon 45**
418	**USH2A** probe 11416-L12142		**Exon 59**
427	Reference probe 14774-L16471	11q23	
436	**USH2A** probe 11417-L12143		**Exon 35**
445	**USH2A** probe 11418-L12144		**Exon 49**
454	Reference probe 10061-L10485	8q22	

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

* New in version A3.

Table 1b. SALSA MLPA Probemix P362-A3 USH2A mix 2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*USH2A*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L13645	5q31	
136	**USH2A** probe 11421-L12147		**Exon 50**
142	**USH2A** probe 11422-L12148		**Exon 40**
148	**USH2A** probe 16839-L19623		**Exon 32**
154	**USH2A** probe 11424-L12150		**Exon 60**
160	Reference probe 12675-L13750	8q22	
166	**USH2A** probe 11425-L12151		**Exon 18**
172	**USH2A** probe 11426-L12152		**Exon 30**
178	**USH2A** probe 11427-L12153		**Exon 62**
184	**USH2A** probe 11428-L12154		**Exon 14**
190	**USH2A** probe 20575-L12157		**Exon 64**
196	**USH2A** probe 11429-L12155		**Exon 66**
202	**USH2A** probe 11430-L12156		**Exon 42**
214	**USH2A** probe 11432-L12158		**Exon 48**
220	Reference probe 16552-L19043	11q13	
226	**USH2A** probe 11433-L12160		**Exon 70**
232	**USH2A** probe 11434-L20182		**Exon 54**
238	**USH2A** probe 11435-L12163		**Exon 68**
244	**USH2A** probe 11436-L12165		**Exon 4**
250 *	Reference probe 17332-L24586	3p21	
256	**USH2A** probe 11437-L12167		**Exon 24**
265	**USH2A** probe 11438-L12168		**Exon 6**
274	**USH2A** probe 16838-L19622		**Exon 22**
283 *	Reference probe 13577-L23176	19p13	
292	**USH2A** probe 11440-L19615		**Exon 36**
301	**USH2A** probe 11441-L12171		**Exon 46**
310	**USH2A** probe 16836-L19620		**Exon 12**
319	**USH2A** probe 20576-L19618		**Exon 72**
330	**USH2A** probe 20577-L12173		**Exon 38**
337	**USH2A** probe 11444-L19619		**Exon 2**
346	Reference probe 10713-L11295	6p12	
358	**USH2A** probe 20578-L19621		**Exon 20**
364	**USH2A** probe 16843-L29091		**Exon 52**
373	**USH2A** probe 11448-L12178		**Exon 10**
382	Reference probe 13329-L14755	18q21	
391	**USH2A** probe 11449-L12179		**Exon 44**
400	**USH2A** probe 16835-L19617		**Exon 58**
409	**USH2A** probe 11451-L12181		**Exon 28**
419	**USH2A** probe 11452-L12182		**Exon 8**
427	Reference probe 13057-L14240	15q14	
436	**USH2A** probe 11453-L12183		**Exon 56**
447	**USH2A** probe 20579-L12184		**Exon 16**
454	**USH2A** probe 11455-L12185		**Exon 34**
463	**USH2A** probe 11456-L12186		**Exon 26**
477	Reference probe 20580-L14666	9q21	

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

* New in version A3.

Table 2. USH2A probes arranged according to chromosomal location

Length (nt) P361 / P362	SALSA MLPA probe	*USH2A* exon[a]	Ligation site NM_206933.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*388-390 (Exon 2)*		
226	11397-L12123	Exon 1	110-111	CGTTGCCAGGCA-ACGAGGGACAGC	0.8 kb
337	11444-L19619	Exon 2	213-214	GTCTTCGCCATT-GCTAAGAACGTC	4.0 kb
244	11400-L12126	Exon 3	1000-1001	TGACACTGGGGA-GAATTCTTGTGA	53.6 kb
244	11436-L12165	Exon 4	1146-1147	GCATCTGGTACT-GTGCAAATAGGA	37.3 kb
136	11385-L12111	Exon 5	1186-1187	TAGAGCAGTTTG-TCGGAAGAATGC	2.2 kb
265	11438-L12168	Exon 6	1355-1356	CATTCCTAATGA-TGCAGGAGACAC	1.3 kb
311	11406-L20628	Exon 7	1666-1667	GAATGAAAAACA-ATGGAGATTTGG	0.5 kb
419	11452-L12182	Exon 8	1748-1749	CAATGTCACATT-TAGCATCCTGAC	1.7 kb
166	11389-L12115	Exon 9	1963-1964	GTCATGCCGATA-ACTGCGACACAA	29.7 kb
373	11448-L12178	Exon 10	2179-2180	CTTTTGAGCACT-TCAGAGGGGGAG	2.8 kb
283	11404-L12130	Exon 11	2267-2268	CTTTTTCCGACA-AGTTGGTGCAGA	38.3 kb
310	16836-L19620	Exon 12	2409-2410	AGGCAGTGCAAT-CAGTGCCAGAAT	3.9 kb
178	11391-L12117	Exon 13	2649-2650	CTCCATGGCTCA-GTGAACAAATTC	15.1 kb
184	11428-L12154	Exon 14	3308-3309	TGTTTGCCAAGA-TGCTTCCATTGC	14.6 kb
366	11411-L20629	Exon 15	3491-3492	GTGTGATGCTTG-TGTTCCCAGTGC	10.1 kb
447	20579-L12184	Exon 16	3594-3595	AGTTCTTCTGCT-ATCAATCTCTCC	7.4 kb
382	11409-L19614	Exon 17	3887-3888	CTCTGTGACACT-TACCTGGACAAC	1.5 kb
166	11425-L12151	Exon 18	4372-4373	CCATCACTGGCT-TGGAGCCATACA	1.7 kb
261	11402-L12128	Exon 19	4494-4495	ATCCCTCCTTCA-GTCTTTCCCCTC	6.4 kb
358	20578-L19621	Exon 20	4659-4660	ACTGCTAAATCC-CAAGAACTATCT	15.0 kb
319	11407-L12133	Exon 21	4913-4914	AGAGTCATCTCT-ACCAGCTCTGAT	78.2 kb
274	16838-L19622	Exon 22	5065-5066	TGATTGTCTTTG-CAGCATCACCTG	8.1 kb
196	11393-L12119	Exon 23	5231-5232	TGCTATTAGGCA-TCAGGCTTTTGG	2.3 kb
256	11437-L12167	Exon 24	5301-5302	AATGGTAGTACT-GTTATTGGAGAT	2.0 kb
154	11388-L12114	Exon 25	5488-5489	AAGAACAAATCA-ACGTGTATAACA	1.2 kb
463	11456-L12186	Exon 26	5588-5589	TATGTTTCATGG-TGGAATGAACTT	5.3 kb
238	11399-L12125	Exon 27	5852-5853	ACTGATGAAGCA-TGCATCGGAGTC	5.0 kb
409	11451-L12181	Exon 28	6053-6054	AGTCAATCTGGA-TGGATGCCTATC	0.3 kb
202	11394-L12120	Exon 29	6191-6192	GATAGCCTCGCA-TGAAGGAGGTTC	2.7 kb
172	11426-L12152	Exon 30	6315-6316	GTGACCTGGGAT-GAACCTGTTGTC	21.6 kb
148	11387-L12113	Exon 31	6511-6512	CTGGCTGTACTG-AGAGCTCACATG	2.1 kb
148	16839-L19623	Exon 32	6690-6691	TATTCAGGCAGT-GAGGAGAACTAC	46.0 kb
391	11413-L12139	Exon 33	6816-6817	CAGCTGCCACCA-GAACACGTGGAT	1.6 kb
454	11455-L12185	Exon 34	7044-7045	ATCAAGCTGGGA-GTAAGTTTATTT	5.8 kb
436	11417-L12143	Exon 35	7174-7175	TCTCCTGGACTG-AGCCTGAATATC	22.3 kb
292	11440-L19615	Exon 36	7281-7282	GGATTTGCTCCT-TGGAGTTTACAT	5.3 kb
256	11401-L12127	Exon 37	7464-7465	AATGGACTCTTA-ACACACTCAGTC	30.6 kb
330	20577-L12173	Exon 38	7571-7572	AGAGACAAACCT-TTGGGTGCTCAT	33.9 kb
301	11405-L12131	Exon 39	7761-7762	TGGTCTACACCA-GCTCGTAATAAC	0.7 kb
142	11422-L12148	Exon 40	7941-7942	GGATTTGGCAGT-GCACATAGTTCT	11.5 kb
268	16840-L29088	Exon 41	8429-8430	GAGTCATTCCAT-GAGGTTTATTGA	9.7 kb
202	11430-L12156	Exon 42	8826-8827	TTACCTACCTAT-GTTACCACTCAC	1.0 kb
208	16841-L19625	Exon 43	8979-8978 reverse	TTTGATGCAAGT-GGCTGCTGGATT	10.8 kb
391	11449-L12179	Exon 44	9153-9154	ACAACGTTAGCT-GGTCTTCCAGAG	21.2 kb
409	11415-L12141	Exon 45	9403-9404	GAGTCCACAGCA-TCAACAGTGCAG	1.4 kb
301	11441-L12171	Exon 46	9475-9476	CAGAGGTTGTCA-TCATCAACAGTA	6.4 kb
342	20574-L20015	Exon 47	9690-9689 reverse	GTGGTAATTTGG-GTTCCATTGCTT	21.0 kb
214	11432-L12158	Exon 48	9889-9890	GTGATGAGCTCT-GCAAGGCAGTGA	3.3 kb
445	11418-L12144	Exon 49	10066-10067	GTGGTGGCCGAA-TACAGGAGGCAC	14.8 kb
136	11421-L12147	Exon 50	10250-10251	GAGGCTTCATGA-TGGCCATGGCCA	8.8 kb
232	11398-L12124	Exon 51	10386-10387	AATATGTCAGAT-ACCATATGCTGC	3.5 kb
364	16843-L29091	Exon 52	10746-10747	ACCATTCATACA-GGGAGTGTAAAC	3.8 kb

Length (nt) P361 / P362	SALSA MLPA probe	*USH2A* exon[a]	Ligation site NM_206933.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
214	11396-L12122	Exon 53	10825-10826	TTTCTGCCTGGA-ACAGCTATGGGC	0.8 kb
232	11434-L20182	Exon 54	11122-11123	GTGCCACCAGTA-GCAAGGTAAGAG	2.0 kb
328	11408-L12134	Exon 55	11164-11165	TTCCGGAGAGCA-TCCTGCCACCAA	13.3 kb
436	11453-L12183	Exon 56	11364-11365	TTCACTCTTACA-GCTTGTACATCT	7.0 kb
142	11386-L12112	Exon 57	11475-11476	ATCAATTCTACA-ACAGTGGAATTA	1.1 kb
400	16835-L19617	Exon 58	11665-11666	GCAGCAGTGCTA-GTGATGATTACA	15.4 kb
418	11416-L12142	Exon 59	11829-11830	CTCAATGATGGA-AGTGTAACACCT	1.8 kb
154	11424-L12150	Exon 60	12027-12028	GCACTGGGGTCA-GCTTGCATAGAG	13.4 kb
274	11403-L12129	Exon 61	12391-12392	ACCGTGTGGTCT-ACCAGGAGAGAC	47.9 kb
178	11427-L12153	Exon 62	12641-12642	GGCATTGCTACT-ACAGTGGTCAGA	4.8 kb
400	11414-L12140	Exon 63	12882-12883	ACTGTCCACTCT-GTGAAGTCCACC	4.3 kb
190	20575-L12157	Exon 64	14331-14332	GCTCCAGGAGGA-TTCCAGCCAACT	20.5 kb
374	11412-L12138	Exon 65	14636-14637	CTCTTCTACCCA-AGCAGTGGTCAA	2.0 kb
196	11429-L12155	Exon 66	14821-14822	GCACCTGCTTCA-ACTGTTGCAGCA	1.0 kb
184	11392-L12118	Exon 67	15004-15005	CTTGCCCTCCTG-ACTCAGCCCTCC	7.0 kb
238	11435-L12163	Exon 68	15230-15231	GTCTGTGGTGTG-TGTGAACTGGAG	1.5 kb
355	11410-L12136	Exon 69	15385-15386	TCTGCACGACTG-ACGAAGGAAGTG	4.6 kb
226	11433-L12160	Exon 70	15518-15519	GCTGTGGTTCAT-AGTGTTAATGGC	5.7 kb
173	11390-L20627	Exon 71	15780-15781	AGTCAAAACCAA-ACCAGCCTAACC	3.1 kb
319	20576-L19618	Exon 72	15965-15966	AGTGACTAAGGA-ACGCACCACATT	
		Stop Codon	*15994-15996 (Exon 72)*		

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

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

References

- 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.
- 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 P361/P362 USH2A

- Dad S et al. (2015). Partial USH2A deletions contribute to Usher syndrome in Denmark. *Eur J Hum Genet.* Dec;23(12):1750. doi: 10.1038/ejhg.2015.131.
- Garcia-Garcia G et al. (2014). Novel deletions involving the USH2A gene in patients with Usher syndrome and retinitis pigmentosa. *Mol Vis.* Sep 25;20:1398-410. eCollection 2014.
- Steele-Stallard et al. (2013). Screening for duplications, deletions and a common intronic mutation detects 35% of second mutations in patients with USH2A monoallelic mutations on Sanger sequencing. *Orphanet J Rare Dis*. 2013; 8: 122.

P361 Product history	
Version	*Modification*
A3	Three reference probes have been replaced.
A2	One reference probe is replaced and the length of some probes have been adjusted.
A1	First release.
P362 Product history	
Version	*Modification*
A3	Two reference probes have been replaced.
A2	The length of several probes has been adjusted.
A1	First release.

Implemented changes in the product description

Version A3/A3-01– 15 July 2019 (02P)

- Product description restructured and adapted to a new template.
- Product description adapted to a new product version.
- Small changes in Table 1 and Table 2.

Version 05 – 06 April 2016 (55)

- Product description adapted to new product versions (version numbers changed, lot numbers added, small changes in Table 1 and Table 2, new pictures included).
- Various minor textual changes.
- Related SALSA MLPA probemix added on page 1.

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