Product Description
SALSA® MLPA® Probemix P433-A2 ARID1A-ARID1B

To be used with the MLPA General Protocol.

Version A2
As compared to version A1, several probes have a change in length but no change in the sequence targeted. For complete product history see page 9.

Catalogue numbers:
- **P433-025R:** SALSA MLPA Probemix P433 ARID1A-ARID1B, 25 reactions.
- **P433-050R:** SALSA MLPA Probemix P433 ARID1A-ARID1B, 50 reactions.
- **P433-100R:** SALSA MLPA Probemix P433 ARID1A-ARID1B, 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 P433 ARID1A-ARID1B is a **research use only (RUO)** assay for the detection of deletions or duplications in the *ARID1A* and *ARID1B* genes, which are associated various with various tumour types, Coffin-Siris syndrome and mental retardation syndrome.

The *ARID1* subfamily contains two members: *ARID1A* (AT-rich interactive domain 1A) and *ARID1B* (AT-rich interactive domain 1B), which share 80% amino acid homology and are evolutionarily conserved. These genes encode proteins for the SWI/SNF chromatin modelling complex. Both ARID1A and ARID1B are able to bind DNA and are suggested to help target the SWI/SNF complexes to the chromatin location that needs to be remodelled.

Chromosomal deletions and mutations of *ARID1A* and *ARID1B* have been identified in 11% of childhood neuroblastoma and are suggested to associate with early treatment failure and decreased survival. Approximately 50% of ovarian clear cell carcinomas carry inactivating mutations in *ARID1A* (Jones et al. 2010, Wiegand et al. 2010). *ARID1A* is located at 1p36.11 chromosomal band, which is suggested to contain several tumour-suppressor genes and, which is also commonly deleted in different tumour types. Frequent *ARID1A* aberrations have been observed in many cancer types, including up to 17% of hepatocellular carcinomas (Guichard et al. 2012, Fujimoto et al. 2012, Huang et al. 2012), 10-29% of gastric cancers (Wang et al. 2011, Zang et al. 2012) and up to 37% of breast cancers (Cornen et al. 2012). Loss of *ARID1A* expression is associated with poor prognosis in urothelial bladder cancer (Balbás-Martínez et al. 2013). Intragenic deletions of *ARID1B* have been detected in 90% of neuroblastoma patient samples (Sausen et al. 2012). *ARID1A* and *ARID1B* aberrations are typically loss-of-function changes; they can exhibit bi-allelic inactivation or loss of protein expression, consistent with a tumour suppressor mechanism (Garraway and Lander, 2013).

Moreover, *ARID1A* and *ARID1B* genes have been implicated in Coffin-Siris syndrome and in mental retardation (OMIM 614607 and OMIM 614562). Both intragenic duplications and microdeletions of *ARID1B* have been described in patients with unexplained intellectual disability (Hoyer et al. 2012), in patients with Coffin-Siris syndrome (Santen et al. 2012, Tsurusaki et al. 2012) and with both syndromic and non-syndromic short stature

(Yu et al. 2015). Also, *ARID1A* microduplications causes intellectual disability with recognizable syndromic features (Bidart et al. 2017).

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 *ARID1A* and *ARID1B* exon numbering used in this P433-A2 ARID1A-ARID1B product description is the exon numbering from the LRG_875 and LRG_861 sequences, respectively. 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 P433-A2 ARID1A-ARID1B contains 56 MLPA probes with amplification products between 127 and 504 nucleotides (nt). This includes 22 probes for the *ARID1A* and 22 probes for *ARID1B* gene. In addition, 12 reference probes are included that detect autosomal chromosomal locations that target relatively copy number stable regions in various cancer types. Complete probe sequences are available online (www.mrcholland.com) and the identity of the genes detected by the reference probes is available in Table 3.

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. 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 for germline analysis should be derived from different unrelated individuals who are from families without a history of Coffin-Siris syndrome and mental retardation, and for tumour analysis from healthy individuals without 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. Samples mentioned in the table below from the Coriell Institute have been tested with this P433-A2 probemix at MRC Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P433-A2	Expected copy number alteration
NA07994	Coriell Institute	6q25.3	*ARID1B*	Heterozygous duplication
NA06802	Coriell Institute	6q25.3	*ARID1B*	Heterozygous deletion

* Indicated 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 P433-A2 ARID1A-ARID1B 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 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 or in or near the *ARID1A* or *ARID1B* genes. 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, the major cause of genetic defects in the *ARID1A* and *ARID1B* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P433 ARID1A-ARID1B.
- 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.

***ARID1A* and *ARID1B* mutation databases**

https://databases.lovd.nl/shared/genes/ARID1A and https://databases.lovd.nl/shared/genes/ARID1B. We strongly encourage users to deposit positive results in the LOVD. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *ARID1A* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P433-A2 ARID1A-ARID1B

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*ARID1A*	*ARID1B*
64-105	Control fragments – see table in probemix content section for more information			
127	Reference probe 18946-L24593	5q31		
134 «	**ARID1A probe** 18899-L24494		**Exon 1**	
142	**ARID1A probe** 18900-L24495		**Exon 7**	
148	**ARID1B probe** 19238-L25319			**Exon 14**
154	**ARID1B probe** 18902-L24497			**Exon 21**
160 « ¥	**ARID1B probe** 18903-L32281			**Exon 1**
166	Reference probe 12741-L21124	21q22		
172 «	**ARID1A probe** 18904-L25189		**Exon 1**	
178	**ARID1A probe** 18905-L24500		**Exon 5**	
184	**ARID1B probe** 18906-L24501			**Exon 16**
190	**ARID1B probe** 18907-L24502			**Exon 19**
196	**ARID1B probe** 18908-L24503			**Exon 10**
202 «	**ARID1B probe** 18909-L24504			**Exon 1**
208	Reference probe 11546-L13329	19q13		
214 ¥	**ARID1A probe** 18910-L32453		**Exon 8**	
220	**ARID1A probe** 18911-L24506		**Exon 2**	
226	**ARID1A probe** 18912-L24507		**Exon 10**	
232 «	**ARID1B probe** 18913-L25477			**Exon 9**
238	Reference probe 08070-L07851	9p13		
244	**ARID1B probe** 18914-L24509			**Exon 6**
250	**ARID1A probe** 18915-L24510		**Exon 4**	
256	**ARID1B probe** 18916-L24511			**Exon 11**
262	**ARID1B probe** 18917-L24512			**Exon 7**
268	**ARID1B probe** 18918-L24513			**Exon 3**
274	Reference probe 17873-L22132	2p21		
281	**ARID1B probe** 18919-L25190			**Exon 13**
287	**ARID1A probe** 18920-L25191		**Exon 16**	
292	**ARID1A probe** 18921-L25192		**Exon 3**	
300	**ARID1B probe** 18922-L24517			**Exon 8**
308	**ARID1A probe** 18923-L24518		**Exon 15**	
316	**ARID1A probe** 18924-L25193		**Exon 19**	
322	**ARID1A probe** 18925-L25194		**Exon 9**	
328	**ARID1A probe** 18926-L25195		**Exon 17**	
337	Reference probe 03959-L20754	11p11		
346	**ARID1A probe** 18927-L24522		**Exon 20**	
353	**ARID1A probe** 18928-L24523		**Exon 12**	
359	**ARID1B probe** 18929-L25196			**Exon 2**
367	Reference probe 15524-L25197	16q13		
373	**ARID1B probe** 18930-L24525			**Exon 15**
380	**ARID1B probe** 18931-L24526			**Exon 5**
391	**ARID1A probe** 18932-L24527		**Exon 14**	
398	**ARID1B probe** 18933-L25198			**Exon 20**
404 ¥	Reference probe 21393-L25199	9q21		
409	**ARID1A probe** 18934-L25200		**Exon 18**	
417	**ARID1B probe** 18935-L25201			**Exon 18**
423	**ARID1B probe** 18936-L25202			**Exon 4**
427	**ARID1A probe** 18937-L25203		**Exon 11**	
436	**ARID1A probe** 18938-L25229		**Exon 13**	
445	Reference probe 13810-L15304	5q14		
451 Ø	**ARID1B probe** 18939-L24534			**Intron 5**
460	**ARID1A probe** 18940-L25206		**Exon 18**	

466	Reference probe 00979-L25207	10p14		
472	**ARID1A probe** 18941-L24536		**Exon 6**	
481	**ARID1B probe** 18942-L24537			**Exon 17**
490	Reference probe 14909-L17529	18p11		
504	Reference probe 09870-L19465	2p15		

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

¥ Changed in version A2. 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.
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. P433-A2 probes arranged according to chromosomal location

Table 2a. *ARID1A*, 1p36.11

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site NM_006015.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*390-392 (ex 1)*		
134 «	18899-L24494	Exon 1	384-385	ACGAGACAGCGG-GGATCATGGCCG	0.6 kb
172 «	18904-L25189	Exon 1	1024-1025	CAACCACCAGTA-CAACTCCTACTA	32.6 kb
220	18911-L24506	Exon 2	1537-1538	GCCATCCAGTCC-AATGGATCAGAT	1.9 kb
292	18921-L25192	Exon 3	2189-2190	TTCCCTCCACCG-CAGGTAAGATAT	1.2 kb
250	18915-L24510	Exon 4	2286-2287	ACCTGAGCCTTC-AGTCAAGACCCT	28.2 kb
178	18905-L24500	Exon 5	2385-2386	GCACATCAGGGA-TTTCCAGCAGCC	0.5 kb
472	18941-L24536	Exon 6	2638-2639	TGCCCAAGATCG-AGGTGAGAGCCT	0.9 kb
142	18900-L24495	Exon 7	25 nt after Exon 7	CCAGTTAGGAGT-AGATACGGGTGA	0.7 kb
214	18910-L32453	Exon 8	2867-2866 reverse	CCAGCCATGCCT-GCACTGGGGTAG	3.3 kb
322	18925-L25194	Exon 9	3182-3183	CCTCCTTATGGA-CAAGGGATTAAT	0.3 kb
226	18912-L24507	Exon 10	3370-3371	GACAGAATCCAA-ATCCAAGGTAGT	1.3 kb
427	18937-L25203	Exon 11	3403-3404	TACTACAACCAA-TGAGAAGATCAC	3.4 kb
353	18928-L24523	Exon 12	3656-3657	AGCAGTGCTGCC-AGCTCCTTGAAA	1.4 kb
436	18938-L25229	Exon 13	3924-3923 reverse	GCCTTACCTCAT-GCCTGGCAATGG	0.4 kb
391	18932-L24527	Exon 14	6 nt after Exon 14	GGAAAGGTGACT-GATCTGATTGCT	0.3 kb
308	18923-L24518	Exon 15	16 nt before Exon 15	TGTTCACCGCTT-GCCTTTCTACGC	0.3 kb
287	18920-L25191	Exon 16	4284-4283 reverse	AGTGCTCATGTT-TCCCTCAGGCCC	0.3 kb
328	18926-L25195	Exon 17	4485-4486	ATCAACAGCAAC-AGCAGGTGAGGA	0.6 kb
409	18934-L25200	Exon 18	4691-4692	TATGGCAATGCC-TATCCTGCCACT	0.5 kb
460	18940-L25206	Exon 18	5204-5205	AGCAAGTCTCCA-TTCCTGCACTCT	0.6 kb
316	18924-L25193	Exon 19	5453-5454	ACATGGGCATTA-GATACCATCAAC	3.4 kb
346	18927-L24522	Exon 20	5546-5547	CTTGTAGAATAT-TTCCGACGATGC	-
		stop codon	*7245-7247 (ex 20)*		

Table 2b. *ARID1B*, 6q25.3

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site NM_001374820.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*304-306 (ex 1)*		
202 «	18909-L24504	Exon 1	1323-1324	CAACATGGCGGA-CAACAAAGCCCC	0.5 kb
160 «	18903-L32281	Exon 1	1848-1849	ATGATGCGGAGC-TACGGCGGCAGC	50.1 kb
359	18929-L25196	Exon 2	2193-2194	CCGTACCCAGGA-GGTTCCTATGGC	42.3 kb
268	18918-L24513	Exon 3	2321-2322	GAAAGAAACATT-CTGGTTGGCAAG	29.9 kb
423	18936-L25202	Exon 4	2472-2471 reverse	CTCACCTGCTGC-GGCTGGTACCTC	34.0 kb

380	18931-L24526	Exon 5	2584-2583 reverse	TCTTACTGGTAA-ACTTGATGGTCT	101.3 kb
451 Ø	18939-L24534	Intron 5	47.8 kb before Exon 6 (NM_001363725.2; 128-129)	GATGCAGCCTTT-GGACTCAAGGTG	48.0 kb
244	18914-L24509	Exon 6	2782-2783	TTGGCTCTCCTG-TAGGAAGCAACC	25.7 kb
262	18917-L24512	Exon 7	2873-2874	GAGCCAGTCAGA-ATCCAGTTCCCA	22.7 kb
300	18922-L24517	Exon 8	21 nt after Exon 8	AAGCTTCTCCAA-AATGCATGGCAG	15.7 kb
232 «	18913-L25477	Exon 9	3369-3370	CCAACTGTGAAC-CGTAAGGCACAG	18.2 kb
196	18908-L24503	Exon 10	3491-3492	CTCTCCCTACAG-CCAGCCCATGAA	7.0 kb
256	18916-L24511	Exon 11	3621-3622	GGTGAATCCAAA-CTGCCCCTGCCT	7.0 kb
281	18919-L25190	Exon 13	3759-3760	GAGCCAGAGAGA-AAGCTCTGGGTC	3.4 kb
148	19238-L25319	Exon 14	4099-4100	AGCCGCCATCTC-CTGGTAAGTGGC	5.3 kb
373	18930-L24525	Exon 15	4170-4171	ATGGCAGAGGTT-CCAGGTGACCTG	0.4 kb
184	18906-L24501	Exon 16	4290-4291	GTGAGTGATTCA-TCCTTCCCGAAA	6.2 kb
481	18942-L24537	Exon 17	4488-4489	ATGTACAACCAA-AGTCCCTCCGGA	2.6 kb
417	18935-L25201	Exon 18	4589-4590	TGGGCAGCAGTA-TCCAGGCCAAGG	2.2 kb
190	18907-L24502	Exon 19	4952-4953	TATGTGGGCAGC-TGAGGGGCCTCA	3.0 kb
398	18933-L25198	Exon 20	5552-5553	TGATGACAGCAC-TGTTGCTACTTT	3.6 kb
154	18902-L24497	Exon 21	6984-6985	GGGGACGCACTA-GCAGCAAGGGCC	-
		stop codon	*7300-7302 (ex 21)*		

[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.

« 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.
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
274	17873-L22132	PPM1B	2p21	AGCAGAAAATCA-TTAGCATTTCCC	02-044,313
504	09870-L19465	PEX13	2p15	TGAGGATGACCA-TGTAGTTGCCAG	02-061,126
445	13810-L15304	ADGRV1	5q14	AAAGGGAGTTTA-GCCTGGCATTAG	05-090,060
127	18946-L24593	IL4	5q31	ATCGACACCTAT-TAATGGGTCTCA	05-132,038
238	08070-L07851	DNAI1	9p13	AGGGATCTGTTT-GCAGTGGGATAT	09-034,491
404	21393-L25199	PCSK5	9q21	GATGAGCTGGAA-TATGATGACGAG	09-078,164
466	00979-L25207	UPF2	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
337	03959-L20754	MYBPC3	11p11	AGGCTGACTACA-GCTTTGTGCCCG	11-047,319
367	15524-L25197	SLC12A3	16q13	CACCCGGAACCT-CAGCCTGATGAT	16-055,478
490	14909-L17529	RNMT	18p11	TACAATGAACTT-CAGGAAGTTGGT	18-013,724
208	11546-L13329	PRPF31	19q13	TGAGCTTACTGA-TCATGATAGGAC	19-059,314
166	12741-L21124	RIPK4	21q22	AAGCCAAGAAGA-TGGAGATGGCCA	21-042,050

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

References

- 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.
- Balbás-Martínez C et al. (2013). ARID1A alterations are associated with FGFR3-wild type, poor-prognosis, urothelial bladder tumors. *PLoS ONE.* 8:e62483.

- Bidart M et al. (2017). Microduplication of the ARID1A gene causes intellectual disability with recognizable syndromic features. *Genet Med.* 19:701-10.
- Cornen S et al. (2012). Mutations and deletions of ARID1A in breast tumors. *Oncogene.* 31:4255-6.
- Fujimoto A et al. (2012). Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet.* 44:760-4.
- Garraway LA and Lander S (2013). Lessons from the cancer genome. *Cell.* 153:17-37.
- Guichard C et al. (2012). Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. *Nat Genet*. 44:694-8.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Hoyer J et al. (2012). Haploinsufficiency of ARID1B, a member of the SWI/SNF-a chromatin-remodeling complex, is a frequent cause of intellectual disability. *Am J Hum Genet*. 90:565-72.
- Huang J et al. (2012). Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. *Nat Genet*. 44:1117-21.
- Jones S et al. (2010). Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science.* 330:228-31.
- Sausen M et al. (2012). Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. *Nat Genet.* 45:12-7.
- Santen GWE et al. (2012). Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin-Siris syndrome. *Nat Genet*. 44:379-80.
- 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.
- Tsurusaki Y et al. (2012). Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. *Nat Genet*. 44:376-8.
- 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.
- Wang K et al. (2011). Exome sequencing identifies frequent mutation of ARID1A in molecular subtypes of gastric cancer. *Nat Genet*. 43:1219-23.
- Wiegand KC et al. (2010). ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med.* 363:1532-43.
- Yu Y. et al. (2015). De novo mutations in ARID1B associated with both syndromic and non-syndromic short stature. *BMC Genomics*. 16:701.
- Zang ZJ et al. (2012). Exome sequencing of gastric adenocarcinoma identifies recurrent somatic mutations in cell adhesion and chromatin remodeling genes. *Nat Genet*. 44:570-4.

Selected publications using SALSA MLPA Probemix P433 ARID1A-ARID1B

- Yu Y. et al. (2015). De novo mutations in ARID1B associated with both syndromic and non-syndromic short stature. *BMC Genomics*. 16:701.

P433 product history	
Version	*Modification*
A2	Several probes have a change in length but no change in the sequence targeted.
A1	First release.

Implemented changes in the product description
Version A2-01 – 27 November 2020 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Various minor textual or layout changes. - Ligation sites of the probes targeting the *ARID1A* and *ARID1B* genes updated according to new version of the NM_006015.6 and NM_001374820.1 reference sequences. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). *Version 4 – 06 February 2020 (T08)* - Gene name *GPR98* has been changed to *ADGRV1* in Table 2c, following HUGO Gene Nomenclature. - Small textual and layout changes. *Version 3 – 27 July 2017 (T08)* - Exon numbering of the ARID1B gene used in product description version 01 has been added to Table 2b. - Minor layout changes. *Version 2 – 21 July 2017 (T08)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Small changes in probe lengths in Table 1 and Table 2 in order to better reflect the true lengths of the amplification product. - New references added on page 2. - Small textual changes throughout the document. - New exon numbering for ARID1B according to LRG_861 in Table 1 and Table 2b. *Version 1 (52)* - Not applicable, new document.

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