Product Description

SALSA® MLPA® Probemix P481-A1 PRKAR1A-ARMC5

To be used with the MLPA General Protocol.

Version A1. For complete product history see page 9.

Catalogue numbers:

- **P481-025R:** SALSA MLPA Probemix P481 PRKAR1A-ARMC5, 25 reactions.
- **P481-050R:** SALSA MLPA Probemix P481 PRKAR1A-ARMC5, 50 reactions.
- **P481-100R:** SALSA MLPA Probemix P481 PRKAR1A-ARMC5, 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 P481 PRKAR1A-ARMC5 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PRKAR1A* and *ARMC5* genes, which are associated with adrenal hyperplasia. *PRKAR1A* is also associated with Carney complex.

PRKAR1A is located on 17q24.2. It encodes a regulatory subunit of the cAMP-dependent protein kinase A (PKA) complex. This subunit inactivates PKA by binding to its catalytic subunits; cAMP causes dissociation of PRKAR1A from the catalytic subunits of PKA, activating them. Phosphorylation mediated by the cAMP/PKA signalling pathway is involved in the regulation of a wide variety of cellular processes. These include metabolism, cell proliferation, differentiation, and apoptosis.

Loss of function mutations in *PRKAR1A* cause Carney complex (CNC, OMIM 160980), an autosomal dominant syndrome characterized by the onset of multiple types of neoplasia. These include endocrine, cardiac, and cutaneous tumours. Adrenocortical tumours in Carney complex often take the form of multiple small cortical pigmented nodules, similar to those in Cushing syndrome. Additionally, translocations resulting in fusion transcripts of *PRKAR1A* have been observed in papillary thyroid carcinoma and acute promyelocytic leukaemia (Romei and Elisei 2012).

ARMC5, located on 16p11.2, is a member of the ARM (armadillo/beta-catenin-like repeat) superfamily, which has been implicated in mediating protein-protein interactions. The ARMC5 protein plays a role in adrenal gland growth homeostasis and corticosteroid synthesis.

Mutations in *ARMC5* are associated with macronodular adrenal hyperplasia (OMIM 615954), an autosomal dominant susceptibility to tumours of the adrenal glands. This often results in clinical Cushing syndrome (Assié et al. 2013). The susceptibility to adrenal tumours has incomplete penetrance, as a somatic second hit to the *ARMC5* gene is required for tumour development.

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=nucleotid
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/

Exon numbering
From description version A1-02 onwards, the *PRKAR1A* and *ARMC5* exon numbering used in this P481-A1 PRKAR1A-ARMC5 product description is the exon numbering from the MANE project (release version 1.0) based on MANE Select transcripts NM_002734.5 and NM_001105247.2, respectively. The *ARMC5* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. 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. Please note that exon numbering for the same gene might be different from literature and in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MLPA Probemix P481-A1 PRKAR1A-ARMC5 contains 49 MLPA probes with amplification products between 130 and 481 nucleotides (nt). This includes 14 probes for *PRKAR1A*, eight probes for *ARMC5,* and 14 probes in the flanking regions of the *PRKAR1A* and *ARMC5* genes. In addition, 13 reference probes are included that target relatively copy number stable regions in various cancer types including adrenal tumours. Probe sequences and the identity of the genes detected by the flanking and reference probes are available in Tables 2 and 3, respectively, 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, derived from germline blood samples, or from tumour tissue and corresponding healthy tissue, 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. Reference samples for germline analysis should be derived from different unrelated individuals who are from families without a history of Carney complex and Cushing syndrome, and for tumour analysis from different healthy individuals without a history of adrenal hyperplasia. 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 have been tested with this P481-A1 PRKAR1A-ARMC5 probemix at MRC Holland and can be used as positive control samples to detect copy number alterations (CNAs) as specified. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA*	Altered target genes in P481-A1	Expected CNA
NA05875	Coriell Institute	16p11.2	*ZNF843*, ***ARMC5***, *TGFB1I1*	Heterozygous deletion
ACC 49 #	DSMZ	17p11.2-q24.2	*FLCN* (17p arm) *BRIP1*, *AXIN2*, *SLC16A6*, *ARSG*, *WIPI1*, ***PRKAR1A***, *FAM20A*	Gain

* Indicated chromosomal bands accommodate genes targeted by MLPA probes. However, the whole extent of CNA present in this cell line cannot be determined by this P481-A1 PRKAR1A-ARMC5 probemix.
# Gain for the reference probe at 250 nt is detected in this sample.

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. When this criterion is 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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 subclonal cases.
- 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 the *PRKAR1A* and *ARMC5* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P481 PRKAR1A-ARMC5.
- 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 and LOVD mutation databases

http://cancer.sanger.ac.uk/cosmic; http://www.lovd.nl. We strongly encourage users to deposit germline positive results in the LOVD database or for somatic mutations in the COSMIC database. 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 *PRKAR1A* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P481-A1 PRKAR1A-ARMC5

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			Location (hg18) in kb
		Reference	***PRKAR1A***	***ARMC5***	
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 19551-L26105	2p13			02-071.750
137	**ARMC5 probe** 22552-L32158			**Exon 1**	16-031.379
143 ¬	Flanking probe 08591-L23693		flanking		17-017.070
148	Reference probe 16544-L19035	11q13			11-070.222
153 ¬	Flanking probe 22166-L31212		flanking		17-063.780
157	**ARMC5 probe** 22551-L31736			flanking	16-031.377
166	Reference probe 12571-L13621	22q13			22-036.837
173 ¬	Flanking probe 22168-L31214		flanking		17-064.376
178 ¬	Flanking probe 21684-L30341		flanking		17-036.042
184	**PRKAR1A probe** 22169-L31215		**Exon 9**		17-064.037
190	Reference probe 09724-L24135	12q24			12-116.149
196	**ARMC5 probe** 22550-L31735			flanking	16-031.378
203	**PRKAR1A probe** 22170-L31216		**Exon 7**		17-064.033
208 ¬	Flanking probe 15771-L18843		flanking		17-060.956
214	Reference probe 22197-L31258	10p14			10-012.019
220 ¬	Flanking probe 22171-L31217		flanking		17-064.063
226	**PRKAR1A probe** 22172-L31218		**Exon 1**		17-064.020
233	**ARMC5 probe** 22549-L31734			**Exon 2**	16-031.381
239	**PRKAR1A probe** 22173-L31219		**Exon 4**		17-064.032
244	**PRKAR1A probe** 22174-L31220		**Exon 11**		17-064.041
250	Reference probe 17580-L31260	7q11			07-073.429
257 ¬	Flanking probe 13980-L31259		flanking		17-065.683
264 ¬	Flanking probe 22175-L32081		flanking		17-063.961
270	**PRKAR1A probe** 22176-L31222		**Exon 10**		17-064.038
276	Reference probe 22198-L08802	10q21			10-055.563
283	**PRKAR1A probe** 22177-L31223		**Exon 8**		17-064.036
292 ¬	Flanking probe 22178-L31224		flanking		17-064.050
301	**PRKAR1A probe** 22179-L31225		flanking		17-064.020
310 ¬	Flanking probe 08568-L08569		flanking		17-057.294
319	Reference probe 17521-L21420	2q32			02-189.563
327	**ARMC5 probe** 22548-L31733			**Exon 6**	16-031.385
335 Ø	**PRKAR1A probe** 22181-L31887		**Intron 1**		17-064.023
348	**PRKAR1A probe** 22182-L31228		**Exon 6**		17-064.033
355	Reference probe 10177-L06584	21q21			21-025.988
361	**PRKAR1A probe** 22183-L32080		**Exon 3**		17-064.030
369 ¬	Flanking probe 06216-L31758			flanking	16-031.393
378	Reference probe 10693-L19115	6p12			06-051.721
385	**PRKAR1A probe** 22184-L31230		**Exon 11**		17-064.038
392	**PRKAR1A probe** 22185-L31231		**Exon 2**		17-064.023
401 ¬	Flanking probe 22186-L31232		flanking		17-063.928
409	**PRKAR1A probe** 22187-L31233		**Exon 5**		17-064.032
418	Reference probe 13817-L31761	2q13			02-108.891
425	**ARMC5 probe** 22547-L31732			**Exon 4**	16-031.383
436 ¬	Flanking probe 22546-L31731			flanking	16-031.356
445	**ARMC5 probe** 22545-L31730			**Exon 3**	16-031.381
456	Reference probe 22555-L31760	11p15			11-017.421
462	**ARMC5 probe** 22544-L31729			**Exon 5**	16-031.385
471 ¬	Flanking probe 20374-L31978			flanking	16-075.071
481	Reference probe 09772-L10187	15q21			15-042.706

[a] See section Exon numbering on page 2 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. P481-A1 probes arranged according to chromosomal location

Table 2a. *ARMC5*

Length (nt)	SALSA MLPA probe	Gene/exon[a]	Location (hg18)/ Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
436 ¬	22546-L31731	*ZNF843*	16**p**11.2	GCGTATCCAAGA-GGATGGACTTAG	21.4 kb
ARMC5, located on 16p11.2. Ligation sites are according to MANE Select transcript NM_001105247.2 (unless otherwise noted).					
157	22551-L31736	upstream (exon 1)	1011 nt before exon 1 (NM_001288767.2; 186-187)	GCTTCTGCCATA-ACCCGGACAACG	0.5 kb
196	22550-L31735	upstream (exon 2)	522 nt before exon 1 (NM_001288767.2; 2 nt after exon 2)	AACGTTCTCGGT-AGGTTTAACCCT	1.0 kb
		start codon	*25-27 (exon 1)*		
137	22552-L32158	Exon 1 (3)	442-443	TAGCCGATTGCT-GTACGGAAGGGG	2.1 kb
233	22549-L31734	Exon 2 (4)	596-597	CTGTGGGGACAT-CCACTGTGCTGG	0.6 kb
445	22545-L31730	Exon 3 (5)	1102-1103	GTCGTGAGGCCA-TCAACCGGGCCC	2.0 kb
425	22547-L31732	Exon 4 (6)	1584-1585	GAGCCTTGGGGA-CGCGAAGGGCCA	1.3 kb
462	22544-L31729	Exon 5 (7)	1895-1896	CACAGGGGAGAG-GCTACTGCAGAA	0.6 kb
327	22548-L31733	Exon 6 (8)	2365-2366	TGCTGTCAGGCA-GCTTTGCAGAAG	7.5 kb
		stop codon	*2830-2832 (exon 6)*		
369 ¬	06216-L31758	*TGFB1I1*	16**p**11.2	CAGGAACTTAAT-GCCACTCAGTTC	43.7 **M**b
471 ¬ #	20374-L31978	*CNTNAP4*	16q23.1	TGGTCCCCTGGA-ACCATTTCTTCT	-

Table 2b. *PRKAR1A*

Length (nt)	SALSA MLPA probe	Gene/exon[a]	Location (hg18)/ Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
143 ¬	08591-L23693	*FLCN*	17**p**11.2	ACCCGGGATATA-TCAGCCATGATA	19.0 **M**b
178 ¬	21684-L30341	*SMARCE1*	17q21.2	TTCACTCCAGAT-TATGATGATGGC	21.3 **M**b
310 ¬	08568-L08569	*BRIP1*	17q23.2	ACGGGTAAGCTT-TATAAGGAAAGT	3.7 **M**b
208 ¬	15771-L18843	*AXIN2*	17q24.1	CCCGAAGCTCTT-GTGAACTGTCTT	2.8 **M**b
153 ¬	22166-L31212	*SLC16A6*	17q24.2	TGGGATACTGCT-TTAGTTTTCTCC	147.4 kb
401 ¬	22186-L31232	*ARSG*	17q24.2	TTCCCTCTCTAG-GTGGAGCCAGGG	32.7 kb
264 ¬	22175-L32081	*WIPI1*	17q24.2	CACGGAAGCAGT-AAGTGTGTGTGA	59.0 kb
PRKAR1A, located on 17q24.2. Ligation sites are according to MANE Select transcript NM_002734.5 (unless otherwise noted).					
301	22179-L31225	upstream (exon 1)	500 nt before exon 1	GCGGGGAGGAGT-CGCCCACCTGTC	0.7 kb
226	22172-L31218	Exon 1 (1b)	41 nt after exon 1	AGGTGAGCTTCG-TCGCTTCGCAGC	2.4 kb
335 Ø	22181-L31887	Intron 1	402 nt before exon 2, reverse (NM_001276289.2; 125-124, reverse)	CAGGGCACAAAA-GTCAACTGTAAG	0.5 kb
		start codon	*126-128 (exon 2)*		
392 #	22185-L31231	Exon 2	263-262, reverse	AGGAATGCCATG-GGTCTCTCAGGT	7.2 kb
361	22183-L32080	Exon 3	4 nt before exon 3	TCGTAATTTCTT-TCAGGAGGAGGC	1.1 kb
239	22173-L31219	Exon 4	559-560	TGATGATAATGA-GAGAAGGTAGGA	0.3 kb
409	22187-L31233	Exon 5	625-626	TGTGATTCAGCA-AGGTAAGGGCCT	0.8 kb
348	22182-L31228	Exon 6	638-639	GGTGATGAAGGG-GATAACTTCTAT	0.8 kb
203	22170-L31216	Exon 7	690-691	ATGTTAACAATG-AATGGGCAACCA	2.1 kb
283	22177-L31223	Exon 8	892-893	AGTCTCTATTTT-AGGTGAGTTGTA	1.0 kb
184	22169-L31215	Exon 9	914-915	GACAAGTGGGAA-CGTCTTACGGTA	1.1 kb
270	22176-L31222	Exon 10	7 nt after exon 10	TTTTGGTATGTA-TGAATTCCCTCA	0.3 kb
385	22184-L31230	Exon 11	1103-1104	TCTCCAGGTGAA-ATTGCACTACTG	3.0 kb
		stop codon	*1269-1271 (exon 11)*		
244	22174-L31220	Exon 11	4076-4077	GTTGTATTCATT-AGTGTATTGGTA	9.5 kb

Length (nt)	SALSA MLPA probe	Gene/exon[a]	Location (hg18)/ Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
292 ¬	22178-L31224	*FAM20A*	17q24.2	CTCTTGCAGGAT-TCTGGACTTCCG	12.8 kb
220 ¬	22171-L31217	*FAM20A*	17q24.2	TGCTGGTAAGGT-TCATGGGATCAA	312.4 kb
173 ¬	22168-L31214	*ABCA8*	17q24.2	TGCCATGTCAGT-GTGGACAGAGGC	1.3 **M**b
257 ¬	13980-L31259	*KCNJ2*	17q24.3	TCTTTCTTCACA-AAGCGGCTCCTG	-

[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.

¬ 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.

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. 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
130	19551-L26105	*DYSF*	2p13	CCATTGCCAAGA-AGGTCAGTGTCC	02-071,750
418	13817-L31761	*EDAR*	2q13	TGGCCAGGTGAA-CCAGCGACAGCA	02-108,891
319	17521-L21420	*COL3A1*	2q32	GTAGACCCGGAC-GACCTGGAGAGC	02-189,563
378	10693-L19115	*PKHD1*	6p12	TTGTACTCATCT-GTTGAATTCAGT	06-051,721
250	17580-L31260	*CLIP2*	7q11	AACAGGAGGTCG-AGAGTTTGCGGG	07-073,429
214	22197-L31258	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
276	22198-L08802	*PCDH15*	10q21	TATTGAAGTGCT-TCCACCAAATAA	10-055,563
456	22555-L31760	*ABCC8*	11p15	TCAAAGGGACCT-GACCCATGACCC	11-017,421
148	16544-L19035	*SHANK2*	11q13	TCTGTGCTGCAA-ATGCTTTGGACC	11-070,222
190	09724-L24135	*NOS1*	12q24	ACCACGGCGTAT-GCTCCTCCTGGC	12-116,149
481	09772-L10187	*SPG11*	15q21	TTTCTTCAGGAT-TGATAGTCATTC	15-042,706
355	10177-L06584	*JAM2*	21q21	TCGTTGTGAAGT-TAGTGCCCCATC	21-025,988
166	12571-L13621	*PLA2G6*	22q13	CTTCCCAATGGA-AGTGGCTTAAGA	22-036,837

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

Related SALSA MLPA probemixes

- P017 MEN1: Contains probes for the *MEN1* gene, mutations in which cause multiple endocrine neoplasia.
- P050 CAH: Contains probes for *CYP21A2* and *CYP21A1P1* genes, which are implicated in congenital adrenal hyperplasia.
- P244 AIP-MEN1-CDKN1B: Contains probes for the *AIP* gene, which is implicated in pituitary adenomas, and for the *MEN1* gene, which is implicated multiple endocrine neoplasia.
- P312 POR: Contains probes for the *POR* gene, which is implicated in congenital adrenal hyperplasia.
- P476 ZNRF3: Contains probes for the *ZNRF3* gene, which is implicated in adrenal hyperplasia.

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Selected publications using SALSA MLPA Probemix P481 PRKA1A-ARMC5

- Giacché M el al. (2023). New pathogenic variants in ARMC5 gene in a series of Italian patients affected by primary bilateral macronodular adrenocortical hyperplasia (PBMAH). *Mol Genet Genomic Med*. 11:e2126.
- Pálla S et al. (2023). Whole genome sequencing resolves 10 years diagnostic odyssey in familiar myxoma. *Sci Rep*. 13:14658.

P481 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 – 27 February 2024 (04P) - Ligation sites and exon numbers of the probes targeting the *PRKAR1A* and *ARMC5* genes updated according to new version of their respective MANE Select NM_ sequences. - Various minor textual or layout changes. Version A1-01 – 09 December 2020 (04P) - Not applicable, new document.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200