Product Description SALSA® MS-MLPA® Probemix ME028-C1 Prader-Willi/Angelman

To be used with the MS-MLPA General Protocol.

Version C1. For complete product history see page 9.

Catalogue numbers:
- **ME028-025R:** SALSA MS-MLPA Probemix ME028 Prader-Willi/Angelman, 25 reactions.
- **ME028-050R:** SALSA MS-MLPA Probemix ME028 Prader-Willi/Angelman, 50 reactions.
- **ME028-100R:** SALSA MS-MLPA Probemix ME028 Prader-Willi/Angelman, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SALSA HhaI (SMR50), and Coffalyser.Net 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.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 MS-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. MRC-Holland cannot provide assistance with data interpretation.

Warning: In several No DNA reactions performed on this ME028-C1 probemix, MRC-Holland has observed a series of non-specific peaks with predominant lengths at about 144, 147, 229, 261, 266, 400 and 409 nucleotides (nt). These peaks appeared very sporadically and we have not yet been able to establish the cause. However, we found that the amount and height of these peaks is greatly reduced by not spinning down your MLPA reactions in between the ligation and PCR reaction. The non-specific peaks are not expected to influence results. Please notify us if you still regularly observe these peaks: info@mlpa.com.

General information: The SALSA MS-MLPA Probemix ME028 Prader-Willi/Angelman is a **research use only (RUO)** assay for the detection of aberrant methylation of one or more sequences of the 15q11 chromosomal region. This probemix can also be used to detect deletions/duplications in the aforementioned chromosomal region.

Genomic imprinting is the monoallelic expression of genes, dependent on the parental origin of the chromosome. It plays a role in growth and development. Imprinting disorders like Prader-Willi syndrome (PWS) and Angelman syndrome (AS) originate from a disturbance in this monoallelic expression by disruption or epimutation of imprinted genes (Ishida et al. 2013).

PWS and AS are distinct neurogenetic disorders, both usually caused by chromosomal deletions on chromosome 15q11 or by uniparental disomy (UPD). In UPD, both copies of a chromosome are inherited from a single parent. The 15q11 chromosomal alterations result in an aberrant expression profile of gene loci that are subject to imprinting. Absence of a paternal allele of chromosome 15q11, due to a chromosomal deletion of (part of) the paternal allele or the presence of two imprinted copies due to maternal UPD, results in PWS. The absence of the maternal copy of the same region or paternal UPD causes AS. Table 4 contains an overview of the expected copy number changes and methylation profiles in PWS/AS patients with deletions or aberrant methylation.

Paternally expressed genes in the 15q11 PWS/AS region are: *MKRN3*, *MAGEL2*, *NDN*, *SNRPN* and the snoRNA cluster; *UBE3A* is only maternally expressed. The PWS-AS Imprinting Centre (IC) (located upstream of the *SNURF-SNRPN* gene) contains both the PWS-SRO (smallest region of deletion overlap) and the AS-SRO. The AS-SRO is required for the PWS/AS region to have the maternal pattern of epigenetic modification and gene expression only if the chromosome has an intact PWS-SRO. The PWS-SRO is a 4.1 kb region that includes the *SNRPN* promoter. The PWS-SRO is unconditionally required for the PWS/AS region to have the paternal pattern of epigenetic modification and gene expression.

Finally, a rare cause of PWS is a small deletion within the SNORD116 cluster, downstream of *SNRPN* (Sahoo et al. 2008). However, these deletions will only be of interest when they are absent in parental samples. Additional probes for the 15q11 region are present in the P343 Autism-1 and the P336 UBE3A probemixes.

Additionally, maternal duplications of the Prader-Willi/Angelman critical region of the 15q11.2-q13.1 region cause the 15q11 duplication syndrome characterized by developmental delay, intellectual disability, hypotonia, and seizures. The extra copy is most commonly a maternal isodicentric 15q11.2-q13.1 supernumerary chromosome (80% of cases) or a maternal interstitial 15q11.2-q13.1 duplication (20% of cases).

The database of genomic variants mentions that copy number changes in the BP1-BP2 (*NIPA1* and *TUBGCP5*) and u1B-u1B* (*SNRPN* exons 1 and 2) regions have been found in healthy individuals (see http://dgv.tcag.ca/dgv/app/home). According to Stefansson et al. (2008), a deletion of the BP1-BP2 region is present in 0.19% of normal individuals and in 0.55% of schizophrenia patients. More probes for this BP1-BP2 region can be found in the P211 HSP region probemix.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1330/ (PWS), https://www.ncbi.nlm.nih.gov/books/NBK1144/ (AS), and https://www.ncbi.nlm.nih.gov/books/NBK367946/ (15q11 Duplication Syndrome).

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/

Probemix content: The SALSA MS-MLPA Probemix ME028-C1 Prader-Willi/Angelman contains 47 (MS−)MLPA probes with amplification products between 129 and 481 nt. Six MS-MLPA probes contain an HhaI recognition site and provide information on the methylation status of the 15q11 chromosomal region. All probes will also give information on copy number changes in the analysed sample. In addition, 11 reference probes are included which are not affected by HhaI digestion and detect genes located outside the 15q11 region. Also, two digestion control probes are included in this probemix that indicate whether or not restriction endonuclease digestion in the MS-MLPA reaction was complete. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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 MS-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 and 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MS-MLPA technique: The principles of the MS-MLPA technique (Nygren et al. 2005, Schouten et al. 2002) are described in the MS-MLPA General Protocol (www.mlpa.com). The MS-MLPA technique should always be internally validated before use in your laboratory. Results of MS-MLPA are highly dependent on the HhaI enzyme used. HhaI enzymes that are resistant to heat inactivation are NOT compatible with the MS-MLPA

technique and will give aberrant results. These include, but may not be limited to, Thermo Fisher Scientific enzymes HhaI, ANZA 59 HhaI, and FastDigest HhaI. We recommend using SALSA HhaI enzyme (SMR50), as this restriction enzyme has been validated for use with MS-MLPA by MRC-Holland.

Required specimens: Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MS-MLPA General Protocol. Additionally, the results of methylation-specific MLPA probes tested on chorionic villi samples (CVS) might not reflect the actual epigenetic constitution of the foetus. This is because the locus of interest might not have reached its final imprinting status in CVS (Paganini et al. 2015). Furthermore, Paganini et al. demonstrated that the rate at which the imprinting is set in foetal tissues may differ between and even within loci. Therefore, only copy number can be determined with ME028 when used on DNA extracted from CVS.

Reference samples: A sufficient number (3 or more) of reference samples should be included in each MS-MLPA experiment for data normalisation and to identify the baseline methylation level for each methylation specific probe. 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 selecting reference samples, please note that methylation patterns may vary between tissues. Reference samples should be derived from unrelated individuals who are from families without a history of PWS or AS. More information regarding the selection and use of reference samples can be found in the MS-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 Institute (https://catalog.coriell.org) and Leibniz Institute 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. Our recommendations for positive control DNA samples are listed below.

Sample ID Coriell biobank	Genotype	Probes affected	Expected DQ	Expected Methylation
NA13556	Female sample: Heterozygous deletion of a small SNRPN region affecting 6 probes; Prader-Willi syndrome.	20694-L28518 to 20687-L28509	0.5	>85%
NA21887	Female sample: Large heterozygous deletion that includes the OCA2 gene and the TUBGCP5/NIPA1 region; Angelman syndrome.	02018-L00865 to 20698-L28526	0.5	0%
NA20375	Male sample: Large heterozygous deletion that includes the OCA2 gene but not the TUBGCP5/NIPA1 region; Angelman syndrome.	20688-L28510 to 20698-L28526	0.5	0%
NA20408	Female sample: Uniparental disomy (no copy number changes); Prader-Willi syndrome.	-	1.0	>85%
NA13554	Male sample: Heterozygous maternally inherited deletion of exon alpha (also named exon u5, or exon 4 in NM_022807.3) of SNRPN affecting 6 probes; asymptomatic, healthy individual.	20694-L28518 to 20687-L28509	0.5	0%
The NIBSC Institute in the U.K. provides an excellent panel of WHO certified genomic DNA samples for Prader-Willi and Angelman syndrome (link). This panel has been described by Boyle et al. (2011).				

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. Reference samples should be consulted to identify base-line methylation levels for each methylation specific probe.

Aberrant methylation of the 15q11 locus: Aberrant methylation of the 15q11 locus can be detected by the five methylation specific MLPA probes detecting sequences in the *SNRPN* and *MAGEL2* genes, when compared to results obtained on DNA samples from healthy individuals.

***SNRPN* locus:** The four methylation specific probes targeting the *SNRPN* gene are located very close to each other. It is expected that all four probes provide similar results. We recommend using the median methylation status of these *SNRPN* probes to determine the methylation status of the *SNRPN* locus and to disregard aberrant methylation detected by a single *SNRPN* MS-MLPA probe.

***MAGEL2* locus:** Imprinting in the 15q11 region outside the *SNRPN* CpG island appears to be not completely established in CVS and foetal cells obtained from amniocytes. Although the *MAGEL2* probe at 232 nt behaves more similar to the *SNRPN* CpG island as compared to the *NDN* probe that was present in ME028 version B, abnormal methylation values for *MAGEL2* are regularly obtained on foetal DNA. **In case of use on DNA derived from foetal tissue it is therefore recommended to disregard the methylation status of the *MAGEL2* locus.**

Interpretation of copy number results: The standard deviation of all probes in the reference samples should be ≤0.10 and the dosage quotient (DQ) of the reference probes 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

Please note that these above mentioned dosage quotients are affected in mosaic samples and foetal samples of dizygotic twin pregnancies. For 15q11 duplication syndrome patients, a duplication in copy number should first be verified before methylation status is assessed. The methylation status in 15q11 duplication patients will not follow the above table. If a duplication is maternally inherited the ratios of imprinted methylation probes are expected to be ~0.7 and if the duplication is paternally inherited the ratios of imprinted methylation probes are expected to be ~0.3.

- 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. 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 (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.

- Digestion Control Probes: The target sequences of the digestion control probes are unmethylated in most blood-derived DNA samples. HhaI-digestion can be considered sufficient when <5% of the signal remains in the digested reaction compared to the undigested reaction.
- We have no data showing that methylation detected by a particular probe indeed influences the corresponding mRNA levels.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Please note that the great majority of Prader-Willi and Angelman samples will show a deletion of 29-33 probes, AND/OR a methylation change of four *SNRPN* and one *MAGEL2* probe. Abnormal copy numbers or methylation changes detected by only one or two probes should be treated with caution.

Limitations of the procedure:

- 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.
- An MS-MLPA probe targets a single specific HhaI site in a CpG island; if methylation is absent for a particular CpG-site, this does not necessarily mean that the whole CpG island is unmethylated!
- Rare cases are known in which an apparent methylation of a methylation sensitive probe proved to be due to a sequence change in or very nearby an HhaI site.
- With this probemix no discrimination between UPD and imprinting defects can be made. For this, it is necessary to perform microsatellite analysis in the patient and parents.

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.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *UBE3A* exons 1 and 3 but not exon 2) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MS-MLPA Probemix ME028-C1 Prader-Willi/Angelman

Length (nt)	SALSA MLPA probe	HhaI site	% methylated in normal blood-derived DNA	% expected signal reduction	Chromosomal position (hg18)			
					Reference	UBE3A	SNRPN	Other 15q
64-105	Control fragments – see table in probemix content section for more information							
129	Reference 18709-L26847				5q31			
137	**OCA2** 20700-L28528							15q13.1
142 Ð	**SNRPN** 20687-L28509	+	50%	50%			CpG island (PWS-SRO)	
148	Reference 08372-L08226				17q12			
154	**TUBGCP5** 02018-L00865						BP1-BP2 region	
160	**UBE3A** 04620-L00863					Exon 3		
166	Reference 08020-L07801				11q24			
172	**MKRN3** 20688-L28510							15q11.2
178	**SNRPN** 04106-L13905	+	50%	50%			CpG island (PWS-SRO)	
184	**UBE3A** 19804-L28512	+	0%	100%		Exon 1		
190	**SNRPN** 04104-L04294	+	50%	50%			CpG island (PWS-SRO)	
195	**UBE3A** 20689-L28513					Exon 2		
202 ¬ ๏	**APBA2** 01314-L00867							15q13.1
208	Reference 07404-L07051				12q13			
214	**SNRPN** 12719-L28514						SNORD116 snoRNA cluster	
220	**GABRB3** 01315-L00868							15q12
226 «	**ATP10A** 20691-L28515							15q12
232	**MAGEL2** 20701-L28529	+	50%	50%				15q11.2
239	**SNRPN** 20692-L15415						Exon u1B*	
244	Reference 08051-L07832				5p15			
250	**SNRPN** 11181-L13997	+	50%	50%			CpG island (PWS-SRO)	
256	**SNRPN** 20694-L28518						Exon u5 (AS-SRO)	
264	Reference 09533-L09943				7q22			
270	**SNRPN** 12182-L28519						Intron u2	
278	**SNRPN** 12179-L13382						Intron u2	
288	**SNRPN** 15261-L16736						Exon u1B	
294	**SNRPN** 01318-L13088						SNURF-SNRPN Exon 3	
301	**UBE3A** 12082-L28520					Exon 4		
310	Reference 14480-L16200				4q12			
317	**OCA2** 20698-L28526							15q13.1
326	**SNRPN** 20697-L28525						SNORD116 snoRNA cluster	
340 #	Digestion control probe 20703-L27783	+	0%	100%	2q12			
347	Reference 18474-L29176				6q24			
355	**UBE3A** 02034-L12925					Exon 9		
364	**ATP10A** 20695-L28523							
373	**UBE3A** 10878-L11548					Exon 1		
382 ๏	**GABRB3** 10874-L11544							15q12
391	**SNRPN** 12477-L13519						Exon U5 (AS-SRO)	
400	Reference 13588-L24039				1q23			
409	**SNRPN** 11177-L28521						SNURF-SNRPN Exon 7	
418 «	**MAGEL2** 11155-L29062							15q11.2
427 «	**NDN** 04026-L29645							15q11.2
436	**NIPA1** 20702-L29063						BP1-BP2 region	
452	Reference 19636-L26295				10p11			
460 #	Digestion control probe 20704-L28531	+	0%	100%	21q22			
472	**SNRPN** 12721-L13796						SNORD116 snoRNA cluster	
481	Reference 19033-L24843				8p21			

Digestion control: warns for insufficient digestion. HhaI-digestion can be considered sufficient when <10% of the signal remains in the digested reaction compared to the undigested reaction.

๏ Be cautious when interpreting results if the probe signal of these two probes in the digested reaction is more than 15% lower than expected. The lower signal in digested reactions of these two probes indicates the use of an excess of HhaI enzyme or the use of an enzyme preparation that is unsuitable for MS-MLPA such as HhaI enzymes that are resistant to heat inactivation.

« 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 outside the common PWS/AS region. The size of the region showing an aberrant copy number will differ between different PWS/AS patients.

Ð This probe is sensitive to overdigestion, which can occur for example in case of lower ligation-digestion temperature (<48°C).

Table 2. ME028-C1 target probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/ Exon	HhaI site	GenBank Ligation site	MV location (hg18)	Imprinted allele	Distance to next probe
436 ‡	20702-L29063	NIPA1		NM_144599.4; 272-273			214.0 kb
154 ‡	02018-L00865	TUBGCP5		NM_052903.4; 897-898			964.5 kb
End of copy number variable region							
172	20688-L28510	MKRN3		NM_005664.3; 1296-1297	15-021,362818		77.5 kb
418 «	11155-L29062	MAGEL2 - Exon 1		NM_019066.4; 3692-3693	15-021,440355		4.1 kb
232	20701-L28529	MAGEL2 - Exon 1	+	NM_019066.4; 369 nt before exon 1, reverse	15-021,444420	Maternal	38.0 kb
427 «	04026-L29645	NDN		NM_002487.2; 1028-1027, reverse	15-021,482490		1137.6 kb
		SNRPN		**NM_022807.3 $**			
288	15261-L16736	U1B		175-176 [Exon 1]	15-022,620016		6.1 kb
239	20692-L15415	U1B*		271-272 [Exon 2]	15-022,626072		64.9 kb
278	12179-L13382	Intron u2		8.2 kb after exon u2 [Exon 3]	15-022,690980		12.3 kb
270	12182-L28519	Intron u2		12.9 kb before exon u5 [Exon 4]	15-022,703328		13.4 kb
256	20694-L28518	u5		376 nt after exon u5 [Exon 4]	15-022,716714		0.6 kb
391	12477-L13519	u5		986 nt after exon u5 [Exon 4]	15-022,717321		33.8 kb
250	11181-L13997	Exon 3 (CpG isl)	+	7.2 kb before exon 3 [Exon 5]	15-022,751105	Maternal	0.1 kb
178	04106-L13905	Exon 3 (CpG isl)	+	7.1 kb before exon 3 [Exon 5]	15-022,751214	Maternal	0.3 kb
190	04104-L04294	Exon 3 (CpG isl)	+	6.8 kb before exon 3 [Exon 5]	15-022,751480	Maternal	0.3 kb
142 Ð	20687-L28509	Exon 3 (CpG isl)	+	6.6 kb before exon 3 [Exon 5]	15-022,751773	Maternal	12.5 kb
294	01318-L13088	Exon 3		726-725 reverse [Exon 6]	15-022,764248		8.3 kb
409	11177-L28521	Exon 7		1089-1090 [Exon 9]	15-022,772555		75.7 kb
214	12719-L28514	SNORD116-1		NR_003316.1; 474 nt after transcript, reverse	15-022,848250		24.4 kb
472	12721-L13796	SNORD116-11		NR_003326.2; 426 nt after transcript	15-022,872658		15.9 kb
326	20697-L28525	SNORD116-23		NR_003337.2; 464 nt after transcript, reverse	15-022,888546		247.8 kb
		UBE3A		**NM_130838.1**			
355	02034-L12925	Exon 9		2368-2369	15-023,136395		20.3 kb
301	12082-L28520	Exon 4		1640-1641	15-023,156677		11.1 kb
160	04620-L00863	Exon 3		614-615	15-023,167740		4.2 kb
195	20689-L28513	Exon 2		94-95	15-023,171919		29.8 kb
373	10878-L11548	Exon 1		33-34	15-023,201674		33.5 kb
184	19804-L28512	Upstream (NM_000462.3, Exon 1)	+	252 kb before exon 1 (53-54)	15-023,235184	N/A	252.8 kb
364	20695-L28523	ATP10A - Exon 15		NM_024490.3; 3235-3236	15-023,487957		171.9 kb
226 «	20691-L28515	ATP10A - Exon 1		NM_024490.3; 494 nt before exon 1	15-023,659906		684.3 kb
220	01315-L00868	GABRB3 - Exon 9		NM_021912.4; 1233-1234	15-024,344242		19.6 kb
382 ๑	10874-L11544	GABRB3 - Exon 7		NM_021912.4; 796-797	15-024,363881		1399.8 kb
137	20700-L28528	OCA2 - Exon 23		NM_000275.2; 2498-2499	15-025,763706		187.1 kb
317	20698-L28526	OCA2 - Exon 3		NM_000275.2; 7 nt after exon 3, reverse	15-025,950765		1246.0 kb
202 ¬ ๑	01314-L00867	APBA2; outside common PWS/AS region		NM_005503.3; 2605-2606	15-027,196749		

$ The exon numbering between square brackets in this column depicts the exon numbering according to the NM sequence for this gene.

๑ Be cautious when interpreting results if the probe signal of these two probes in the digested reaction is more than 15% lower than expected. The lower signal in digested reactions of these two probes indicates the use of an excess of HhaI enzyme or the use of an enzyme preparation that is unsuitable for MS-MLPA such as HhaI enzymes that are resistant to heat inactivation.

« 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 outside the common PWS/AS region. The size of the region showing an aberrant copy number will differ between different PWS/AS patients.

‡ The chromosomal order of these probes in the NCBI and BLAT databases (hg18) is different. We used the order that is mentioned by Jiang et al. (2008).

Ð This probe is sensitive to overdigestion, which can occur for example in case of lower ligation-digestion temperature (<48°C).

Note: The exon numbering used in this ME028-C1 Prader-Willi/Angelman product description is the exon numbering from the RefSeq transcripts: NG_016776.1 (NM_019066.4; *MAGEL2*), LRG_15 (NM_130838.1; *UBE3A*), NG_009282.1 (NM_024490.3; *ATP10A*), NG_012836.1 (NM_021912.4; *GABRB3*) and NG_009846.1 (NM_000275.2; *OCA2*). The exon numbering used for *SNRPN* is based on the exon numbering commonly used in the literature. The exon numbering and NM sequences used are from 05/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 3. Methylation specific target probe sequences detected by ME028-C1

Length (nt)	SALSA MLPA probe	Partial sequence with HhaI site
142	20687-L28509	CGGTATTTAGGGGGTGTTGA**GCGC**AGGT-AGGTGTATAATAGTGACCACTGCGTGGTGGA
178	04106-L13905	CCGCTGCTGCAGCGAGTCTG**GCGC**AGAGT-GGAGCGGCCGCCGGAGATGCCTGACGCATC
184	19804-L28512	CTAGCCGCGAGATCCGTGTGTCTCCCAAGA-TGGTG**GCGC**TGGGCTCGGGGTGACTACAGGA
190	04104-L04294	CACCGATGGTATCCTGTCCGCTCGCAT-TGGG**GCGC**GTCCCCCATCCGCCCCCAACTGTGGT
232	20701-L28529	TCTGGGCTAAGATGTGAGGCGGAGAATGAA-AAAA**GCGC**ATTTACATAAGAGAGTTCCAGG
250	11181-L13997	GGAGGGAGCTGGGACCCCTGCA-CTGCGGCAAACAAGCACGCCT**GCGC**GGCCGC

The HhaI sites are marked in **grey**. Ligation sites are marked with -.

Table 4. Interpretation of copy number and methylation ratio results

	PWS Deletion	PWS Disomy ¥	Reference	AS Disomy ¥	AS Deletion	Duplication
Genomic situation of the 15q11 region *	M_	MM	PM	PP	P_	PMM
Copy number	1	2	2	2	1	3
Copy number ratio	0.5	1	1	1	0.5	1.5
% Methylated	100%	100%	50%	0%	0%	70%
Ratio after digestion	1	1	0.5	0	0	0.7

* In this row, the paternal and maternal copies of the 15q11 region are indicated with a P or M, respectively.

¥ Next to uniparental disomy, PWS/AS can also be caused by aberrant methylation due to imprinting defects. With the ME028 probemix no discrimination between uniparental disomy and imprinting defects can be made.

Related SALSA MLPA probemixes

- ME030 BWS/RSS: Contains probes for Beckwith-Wiedemann syndrome.
- ME031 GNAS: Contains probes for the complex GNAS region on chromosome 20.
- ME032 UPD7-UPD14: Uniparental disomy of chromosomes 7 and 14.
- ME033 TNDM: Probes for the transient neonatal diabetes mellitus (TNDM) region on 6q24.
- ME034 Multi-locus Imprinting: Probes for eleven different imprinted locations.
- P211 HSP region: Contains additional probes for the BP1-BP2 region.
- P325 OCA2: Contains probes for each exon of *OCA2* with the exception of exon 8.
- P336 UBE3A: Contains probes for each *UBE3A* exon.
- P343 Autism-1: Contains additional probes for *UBE3A* and the 15q13 region.

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ME028 Product history	
Version	*Modification*
C1	The methylation sensitive *NDN* probe has been replaced by a *MAGEL2* probe. Two probes for the *OCA2* gene have been included. Four other 15q11 probes, the two digestion control probes and several reference probes have been replaced.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	A large number of new 15q11 probes have been included in version B1.
A1	First release.

Implemented changes in the product description

Version C1-04 – 06 September 2019 (01M)
- Advice on not spinning down your MLPA reactions in between the ligation and PCR reaction was added to the warning about non-specific peaks on page 1.
- Catalogue number SALSA HhaI adjusted.

Version C1-03 – 24 January 2019 (01M)
- Exon number changed for positive sample NA13554.

Version C1-02 – 17 December 2018 (01M)
- Use of SALSA HhaI (SMR51) with ME028 added.
- Information regarding 15q11 duplication syndrome added.
- Additional positive control samples included.

- Warning added under copy number status table.
- Digestion control warning updated: removed signal should be gone upon complete digestion and added HhaI-digestion can be considered sufficient when <5% of the signal remains in the digested reaction compared to the undigested reaction.
- Order of *TUBGCP5* and *NIPA1* switched in Table 2.
- Old exon numbering for *UBE3A* removed from Table 2.
- Warning added regarding overdigestion of the 142 nt probe.
- Example of duplication added to Table 4.
- Figure 4 removed.
- ME034 added as a related probemix.
- References updated.

Version C1-01 – 14 June 2018 (01M)
- Product description restructured and adapted to a new template.
- Various minor textual and layout changes.
- Table 3 with methylation specific target probe sequences detected by ME028-C1 added.
- P211 HSP region added to the related probemixes.

Version 59 – 5 January 2018 (16)
- Warning added in Table 1 and Table 2, 226 nt probe 20691-L28515.

Version 58 – 10 August 2017 (16)
- Link to NIBSC panel updated.
- Various minor textual changes.

Version 57 – 08 June 2017 (16)
- Notification regarding the methylation status of CVS samples added under Methylation-specific MLPA section.

Version 56 – 23 March 2017 (15)
- Ligation site information of SNRPN in table 2 adapted to NM_022807.3.

Version 55 – 23 December 2016 (15)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Various textual changes on page 1.
- Warning on the type of non-specific peaks included on page 1.

Version 54 – 07 December 2016 (15)
- Warning regarding HhaI enzymes that are resistant to heat inactivation added under Methylation-specific MLPA section.

Version 53 – 14 January 2016 (14)
- Product description adapted to a new lot (lot number added, new pictures included).
- Manufacturer's address adjusted.
- New references added on page 2.
- Various minor textual changes.
- UBE3A exon numbering is changed.
- Warning about SNP influencing the signal of the 287 nt probe added to Table 1 and 2.

Version 52 – 22 July 2015 (13)
- Section about the interpretation of results expanded and clarified, including a new table and figure.
- Various minor textual changes.
- Various minor layout changes.

Version 51 (12)
- Extra information about genetic region of certain probes (PWS-SRO) added to Table 1 and 2.

Version 50 (12)
- Error corrected in description of digestion control probes.
- Various minor textual changes.

Version 49 (08)
- This product description has been changed to incorporate a new lot (lot number added, new picture included).

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