Product Description SALSA® MLPA® Probemix P343-C3 Autism-1

To be used with the MLPA General Protocol.

Version C3. For complete product history see page 7.

Catalogue numbers:
- **P343-025R:** SALSA MLPA Probemix P343 Autism-1, 25 reactions.
- **P343-050R:** SALSA MLPA Probemix P343 Autism-1, 50 reactions.
- **P343-100R:** SALSA MLPA Probemix P343 Autism-1, 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.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 P343 Autism-1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the 15q11-q13 chromosomal region (including *UBE3A*, *GABRB3* and the 15q13 microdeletion region with *CHRNA7*), the 16p11 microdeletion region and the *SHANK3* gene at 22q13, which are associated with autism.

Multiple studies postulate that at least some autism cases have a genetic basis and many different loci have been implicated in autism. This P343-C3 probemix contains MLPA probes for three of these chromosomal regions: the 15q11-q13 chromosomal region, the 16p11 microdeletion region and the *SHANK3* gene. The TRPM1, KLF13 and CHRNA7 probes are located within the common 15q13 microdeletion region that has been described by Sharp et al. (2008). Please note that 15q13 duplications were identified not only in 12 out of 1223 epilepsy patients but also in 23 out of 3699 control samples (Helbig et al. 2009). 15q13 deletions were identified in 12 out of 1223 individuals with idiopathic generalized epilepsy and in 9 out of 3391 schizophrenia patients (International Schizophrenia Consortium 2008). No 15q13 deletions were detected in 3181 control samples.

Genomic imbalances of an approximately 600 kb region in 16p11.2 (29.5-30.1 Mb) have been associated with autism, intellectual disability, congenital anomalies, and schizophrenia. A recurrent microdeletion syndrome on 16p11.2-p12.2 has been described by Ballif et al. (2007). The phenotype included developmental delay. The size of the deletion is different in the five subjects described, however, all included the *PALB2* and *IL21R* genes.

Please note that 15q11, 15q13 and 16p11.2 deletions and duplications have also been described in healthy individuals. Phenotype prediction for abnormalities detected in these regions is very difficult. The great majority of the probes targeting the 15q11 region differ from the probes present in the ME028 Prader-Willi-Angelman probemix. This P343 probemix may therefore also be useful for further characterisation of large deletions in Prader-Willi/Angelman patients.

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 *UBE3A, ATP10A, GABRB3, OCA2* and *SHANK3* exon numbering used in this P343-C3 Autism-1 product description is the exon numbering from the RefSeq transcripts NM_130838.4, NM_024490.3, NM_021912.5, NM_000275.3 and NM_001372044.2, respectively. The *SHANK3* and *UBE3A* exon numbering has changed. From description version C3-01 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for these genes. The exon numbering used in previous versions of this product description can be found in between brackets in Table 2. 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 Probemix P343-C3 Autism-1 contains 50 MLPA probes with amplification products between 121 and 500 nucleotides (nt). Within the 15q11 region, this includes two probes for the SNRPN-HB2-85 cluster, five probes for the *UBE3A* gene, two probes for *ATP10A*, seven probes for *GABRB3* and two probes for *OCA2*. In addition, nine probes are present detecting 15q13 sequences, including three probes that are located within the common 15q13 microdeletion region. The 16p11.2 region is covered by 11 probes detecting sequences in the 28.9-30.2 Mb region. Three probes are included for the *SHANK3* gene (exons 4, 15, and 22). In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.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 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, 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 autism. 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 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.

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 *SHANK3* 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.

P343 specific note:

- The *SHANK3* gene is located in an extremely GC-rich chromosomal area, 49 Mb from the p-telomere of chromosome 22. Many SHANK3 probes have a higher than average standard deviation in many of our tests. Apparent deletions and duplications observed by only one or two of these 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.

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.

***UBE3A* mutation database:** https://databases.lovd.nl/shared/genes/UBE3A
***ATP10A* mutation database:** https://databases.lovd.nl/shared/genes/ATP10A
***GABRB3* mutation database:** https://databases.lovd.nl/shared/genes/GABRB3
***OCA2* mutation database:** https://databases.lovd.nl/shared/genes/OCA2
***SHANK3* mutation database:** https://databases.lovd.nl/shared/genes/SHANK3

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

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *GABRB3* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P343-C3 Autism-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	15q11-q13	16p11	SHANK3
64-105	Control fragments – see table in probemix content section for more information				
121	Reference probe 19616-L27455	4p13			
130	Reference probe 08640-L08656	3q26			
136	**ATP10A probe** 12964-L14669		15q12 **Exon 1**		
142	**UBE3A probe** 10883-L11553		15q11 **Exon 6**		
148 ±	**GABRB3 probe** 10872-L11542		15q12 **Exon 6**		
154	Reference probe 09431-L09680	11q13			
160	**UBE3A probe** 04620-L14668		15q11 **Exon 4**		
166	**KLF13 probe** 08376-L08230		15q13 **Exon 2**		
172	**HIRIP3 probe** 11667-L14670			**Exon 3**	
178	**NDNL2 probe** 08377-L08231		15q13 **Exon 1**		
184	**GABRB3 probe** 10868-L11538		15q12 **Exon 4**		
190	Reference probe 20256-L23585	12q12			
197	**UBE3A probe** 10880-L11550		15q11 **Exon 3**		
202	**APBA2 probe** 01314-L00867		15q13 **Exon 14**		
208	**SEZ6L2 probe** 11668-L12439			**Exon 1**	
214	**SNRPN-HB2-85 probe** 21014-L29483		15q11		
220	**GABRB3 probe** 01315-L09339		15q12 **Exon 9**		
226	**DOC2A probe** 13162-L12447			**Exon 4**	
232 «	**SHANK3 probe** 06787-L07383				**Exon 22**
238 «	**MAZ probe** 11669-L12440			**Exon 5**	
244	**UBE3A probe** 10886-L14677		15q11 **Exon 9**		
250	Reference probe 02658-L02125	11q22			
256	**UBE3A probe** 01317-L12925		15q11 **Exon 10**		
264	Reference probe 08874-L19215	1p31			
270	**ATP10A probe** 11165-L12883		15q12 **Exon 16**		
286	**CHRNA7 probe** 12956-L08237		15q13 **Exon 4**		
292	**GABRB3 probe** 10875-L11545		15q12 **Exon 8**		
300 «	**TJP1 probe** 08389-L14671		15q13 **Intron 1**		
310 «	**SHANK3 probe** 20567-L14007				**Exon 4**
319	**GABRB3 probe** 10870-L11540		15q12 **Exon 5**		
328	Reference probe 07631-L07316	10q26			
337	**CD2BP2 probe** 11671-L12442			**Exon 4**	
346	**MVP probe** 00550-L22423			**Exon 5**	
355	**GABRB3 probe** 10867-L11537		15q12 **Exon 3**		
364	**SPN probe** 11672-L12443			**Exon 3**	
373	**TRPM1 probe** 08397-L14672		15q13 **Exon 27**		
382	**GABRB3 probe** 10874-L11544		15q12 **Exon 7**		
391 «	**SHANK3 probe** 14190-L15800				**Exon 15**
400	Reference probe 15766-L24901	14q32			
409	Reference probe 07208-L06858	7p14			
420 «	**MAZ probe** 11673-L29557			**Exon 6**	
427	**SCG5 probe** 12951-L29660		15q13 **Exon 6**		
436	**OCA2 probe** 02040-L01553		15q12-13 **Exon 22**		
445	**OCA2 probe** 02041-L03725		15q12-13 **Exon 1**		
454	**HIRIP3 probe** 11674-L12445			**Exon 4**	
465	**MAPK3 probe** 11675-L12446			**Exon 5**	
475	**SNRPN-HB2-85 probe** 12720-L13795		15q11		
483	**LAT probe** 11677-L12448			**Exon 4**	
492	**SCG5 probe** 12954-L14464		15q13 **Exon 3**		
500	Reference probe 10218-L14675	7q22			

± SNP rs75015217 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.

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

Table 2. P343-C3 probes arranged according to chromosomal location

Table 2a. 15q11-15q13 region

Length (nt)	SALSA MLPA probe	Gene NM_sequence / Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
214	21014-L29483	SNRPN-HB2-85		AAGAAATCCCTT-CCAGGAGGGCTC	10.2 kb
475	12720-L13795	SNRPN-HB2-85		AAGTTCTTTAAC-GTCATCGGCTTG	277.9 kb
UBE3A gene (NM_130838.4). *CDS: 577-3135*					
256	01317-L12925	Exon 10 (9)	2900-2901	TCATTCATTTAC-AGATGAACAGAA	14.2 kb
244	10886-L14677	Exon 9 (8)	2868-2869	TCTGTTCTGATT-AGGTGAGGTACT	2.4 kb
142	10883-L11553	Exon 6 (5)	2428-2429	TCTACAGGAAGC-TAATGGGGAAAA	14.8 kb
160	04620-L14668	Exon 4 (3)	1146-1147	TCTTCCTCAAGG-ATAGGTGATAGC	4.2 kb
197	10880-L11550	Exon 3 (2)	626-627	CTACCACCAGTT-AACTGAGGGCTG	312.1 kb
ATP10A gene (NM_024490.3). *CDS: 107-4606*					
270	11165-L12883	Exon 16	3303-3304	TGCAGTGCCGAA-ATTCCGATACCT	175.0 kb
136	12964-L14669	Exon 1	375-376	GGCCAACGTGTA-CTTTGTCTTCAT	685.2 kb
GABRB3 gene (NM_021912.5). *CDS 40-1461*					
220	01315-L09339	Exon 9	1208-1209	CGATACCAGGAA-TTCAGCAATATC	13.1 kb
292	10875-L11545	Exon 8	10 nt before exon 8	CACCACTTTGTT-TCTTTTCTAGGG	6.5 kb
382	10874-L11544	Exon 7	771-772	AGGAACATTGGA-TACTTCATTCTT	12.6 kb
148 ±	10872-L11542	Exon 6	18 nt after exon 6	CCTGCATCCACT-TATAGTCCCTTC	3.1 kb
319	10870-L11540	Exon 5	29 nt before exon 5	CAGCCCTTCTTT-AATATCTTCCCT	38.0 kb
184	10868-L11538	Exon 4	351-352	GGGATCCCTCTC-AACCTCACGCTT	151.0 kb
355	10867-L11537	Exon 3	223-224	GTCCCCCGGTCT-GCGTGGGGATGA	1.2 **M**b
OCA2 gene (NM_000275.3). *CDS 114-2630*					
436	02040-L01553	Exon 22	2423-2424	CCGCTCATGTAT-GCCCTGGCCTTC	247.8 kb
445	02041-L03725	Exon 1	91-90 reverse	TGCACTTTACCT-GCGCACTTGCAG	1.2 **M**b
202	01314-L00867	*APBA2*		CACCACCCACTT-GATTTTTTTCAT	152.0 kb
178	08377-L08231	*NDNL2*		CTCTTGGGTTCA-AGTTCCACCAGC	552.2 kb
300 «	08389-L14671	*TJP1*		CACAGGCTGAGT-GGAGTGTTTTGC	1.2 **M**b
373	08397-L14672	*TRPM1*		ATGGACATCCTA-GGAATGTGAAAT	370.6 kb
166	08376-L08230	*KLF13*		TTGAACCCCCTT-TCTCAGGGATGG	739.3 kb
286	12956-L08237	*CHRNA7*		AGACTGTTCGTT-TCCCAGATGGCC	568.0 kb
SCG5 gene (NM_1144757.2). *CDS 174-812*					
492	12954-L14464	Exon 3	477-478	TGACTGGAGACA-ACATTCCTAAGG	16.8 kb
427	12951-L29660	Exon 6	865-866	TCAGCATGGCTT-ATGTGCACGTGT	

Table 2b. 16p11 region

Length (nt)	SALSA MLPA probe	Gene detected	Partial sequence[b] (24 nt adjacent to ligation site)	Location (hg18) in kb	Distance to next probe
		p-telomere			
483	11677-L12448	LAT	ACCAGTTTGTAT-CCAAGGGGCATC	16-028.905	678.2 kb
364	11672-L12443	SPN	CCATCAAGATGT-CATCAGTGCCCC	16-029.583	145.5 kb
238 «	11669-L12440	MAZ exon 5	CCACGGCAGCAT-ACCTGCGCATCC	16-029.728	0.8 kb
420 «	11673-L29557	MAZ exon 6	GAAGAAATGTTT-TCTTAGGGGAAT	16-029.729	23.6 kb
346	00550-L22423	MVP	GTCGTGGAGATC-ATTCAGGCCACC	16-029.753	65.1 kb
208	11668-L12439	SEZ6L2	GCAGCCAGATTA-CTTAGAGAGGCA	16-029.818	95.7 kb
454	11674-L12445	HIRIP3 exon 4	GGCGAGCCTCAA-AGGCAGTTGAGG	16-029.914	0.5 kb
172	11667-L14670	HIRIP3 exon 3	CCAGGGAAGACA-AACTGGACCTTA	16-029.914	14.0 kb
226	13162-L12447	DOC2A	CACTTGCTGCCT-GGAGCCTGTAAG	16-029.928	108.5 kb
465	11675-L12446	MAPK3	CTGGATCAGCTC-AACCACATTCTG	16-030.036	236.3 kb
337	11671-L12442	CD2BP2	GGAAGGCCACTT-TGATGCCGATGG	16-030.273	
		centromere			

Table 2c. *SHANK3* gene

Length (nt)	SALSA MLPA probe	SHANK3 exon[a]	Ligation site NM_001372044.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*371-373 (Exon 2)*		
310 «	20567-L14007	Exon 4 (3)	883-884	AAGCGGCGAGTT-TATGCCCAGAAC	27.6 kb
391 «	14190-L15800	Exon 15 (14)	2344-2345	GAGGGCTTTGGT-TTTGTGCTCCGG	18.1 kb
232 «	06787-L07383	Exon 22 (21)	5076-5077	ACCAACTGTGAT-CAGTGAGCTCAG	
		stop codon	*5789-5791 (Exon 23)*		

± SNP rs75015217 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.

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.

Related SALSA MLPA probemixes

ME028 PWS/AS	Contains probes to detect Prader-Willi and Angelman syndrome (15q region).
P188 22q13	Broad screening of the 22q13 region.
P325 OCA2	Contains probes for nearly all *OCA2* exons.
P336 UBE3A	Contains probes for all *UBE3A* exons.
P339 SHANK3	Contains probes for all *SHANK3* exons

References

- Ballif BC et al. (2007). Discovery of a previously unrecognized microdeletion syndrome of 16p11.2–p12.2. *Nature Genetics* 39:1071-1073.
- Helbig I et al. (2009). 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nature Genetics 41:160-162.*
- International Schizophrenia Consortium (2008). Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature* 455: 237–241.
- 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.
- Sharp AJ et al. (2008). A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures. *Nature Genetics* 40:322-328.
- 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 P343 Autism-1

- Moreira DP et al. (2014). Investigation of 15q11-q13, 16p11.2 and 22q13 CNVs in autism spectrum disorder Brazilian individuals with and without epilepsy. *PloS one* 9(9):e107705.
- Rodriguez-Lopez J et al. (2015). An efficient screening method for simultaneous detection of recurrent copy number variants associated with psychiatric disorders. *Clinica Chimica Acta* 445:34-40.
- Moreira ES et al. (2016). Detection of small copy number variations (CNVs) in autism spectrum disorder (ASD) by custom array comparative genomic hybridization (aCGH). *Res Autism Spectr Disord* 23:145-151.
- Szczałuba K et al. (2016). Paternally Inherited GABRB3 Intragenic Deletion in a Boy with Autistic Features and Angelman Syndrome Phenotype Case Report and Literature Review. *Autism-Open Access* 1-4.

P343 Product history	
Version	*Modification*
C3	Five reference probes have been replaced, one reference probe has been added and the lengths of several probes have been adjusted.

C2	QDX2 control fragments have been added.
C1	TJP1 and SHANK3 probes (391, 400) have been replaced by two new SHANK3 probes.
B1	Several reference and target probes replaced.
A1	First release.

Implemented changes in the product description
Version C3-02 — 27 March 2025 (02P) - Exon numbering, NM_reference sequence and ligation sites of the probes targeting the *UBE3A* gene have been changed. *Version C3-01 — 07 October 2020 (02P)* - Product description rewritten and adapted to a new template. - Exon numbering of the *SHANK3* gene has been changed. - Ligation sites of the probes targeting the *GABRB3, OCA2, SCG5 and SHANK3* genes updated according to new version of the NM_ reference sequence. - Warning added to Table 1 and 2: SNP rs75015217 could influence signal of probe 10872-L11542. - Added LOVD Database references for *UBE3A, ATP10A, GABRB3, OCA2* and *SHANK3* genes.

More information: www.mlpa.com; www.mlpa.eu	
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