Product Description
SALSA® MLPA® Probemix P343 Autism-1

To be used with the MLPA General Protocol.

Version C3
For complete product history see page 9.

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

SALSA® MLPA® Probemix P343 Autism-1 (hereafter: P343 Autism-1) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P343-025R	**P343-050R**	**P343-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding 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
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 product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE

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_130839.5 (MANE), NM_024490.3, NM_000814.6 (MANE), NM_000275.3 and NM_001372044.2, respectively. The *UBE3A* and *GABRB3* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 2. From description version C3-01 onwards, we have adopted the NCBI exon numbering that is present in the MANE Select NM_ sequence for these genes. 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
P343 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.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 MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from 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 (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. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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/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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net 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.

- 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. Sequence changes (e.g. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) 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 *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: https://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 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.

P343 Autism-1 specific note(s):

- 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. 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.

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 in sequence data 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.

Mutation databases
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 https://varnomen.hgvs.org.

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

Table 1. P343-C3 Autism-1

Length (nt)	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 8**		
148 ±	**GABRB3 probe** 10872-L11542		15q12	**Exon 6**		
154	Reference probe 09431-L09680	11q13				
160	**UBE3A probe** 04620-L14668		15q11	**Exon 6**		
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 5**		
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 11**		
250	Reference probe 02658-L02125	11q22				
256	**UBE3A probe** 01317-L12925		15q11	**Exon 12**		
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				

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

± SNV 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.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. Target and flanking probes arranged according to chromosomal location

Table 2a. 15q11-15q13 region

Length (nt)	MLPA probe	Gene NM_sequence / exon[a]	Ligation site[b]	Partial sequence[c] (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_130839.5). *CDS: 701-3319*				
256	01317-L12925	Exon 12 (10)	3084-3085	TCATTCATTTAC-AGATGAACAGAA	14.2 kb
244	10886-L14677	Exon 11 (9)	3052-3053	TCTGTTCTGATT-AGGTGAGGTACT	2.4 kb
142	10883-L11553	Exon 8 (6)	2612-2613	TCTACAGGAAGC-TAATGGGGAAAA	14.8 kb
160	04620-L14668	Exon 6 (4)	1330-1331	TCTTCCTCAAGG-ATAGGTGATAGC	4.2 kb
197	10880-L11550	Exon 5 (3)	810-811	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_000814.6). *CDS 105-1526*				
220	01315-L09339	Exon 9	1273-1274	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	836-837	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	416-417	GGGATCCCTCTC-AACCTCACGCTT	151.0 kb
355	10867-L11537	Exon 3	288-289	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)	MLPA probe	Gene detected	Partial sequence[c] (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)	MLPA probe	SHANK3 exon[a]	Ligation site[b] NM_001372044.2	Partial sequence[c] (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)*		

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNV 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.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related products
For related products, see the product page on our website.

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 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 C3-03 — 22 December 2025 (05P)
- Product description adapted to a new template.
- Exon numbering, NM_reference sequence and ligation sites of the probes targeting the *UBE3A* gene have been updated to the MANE in section: Exon numbering and Table 1 & 2, no change in actual target sites.
- NM_reference sequence and ligation sites of the probes targeting the *GABRB3* gene have been updated to the MANE in section: Exon numbering and Table 2, no change in actual target sites.
- Related SALSA MLPA products section replaced with reference to product page on website.

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.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