SALSA MLPA probemix P336-A1 UBE3A

Lot A1-0909.

Angelman syndrome (AS) is a neurogenetic disorder characterized by severe mental retardation, ataxia, seizures, EEG abnormalities and bouts of inappropriate laughter. AS individuals fail to inherit a normal active maternal copy of ubiquitin protein ligase E3A (UBE3A). UBE3A is subject to genomic imprinting, with predominant transcription of the maternal allele in brain. The known genetic causes of AS are maternal deletion of chromosome 15q11-q13, paternal chromosome 15 uniparental disomy, UBE3A mutations and 15q11 imprinting defects. Maternal duplications of 15q11-q13 lead to a distinct condition that often includes autism (Hogart *et al.*, 2009). Mimicking conditions for AS are found in MTHFR gene. Homozygosity for the MTHFR 677C>T variant (A222V) might increase the risk of a maternal imprinting defect (Williams *et al.*, 2001, Zogel *et al.*, 2006). There has been some evidence for linkage of 16p13 to autism (IMGSAC 2001)

The UBE3A gene (14 exons) spans ~102 kb of genomic DNA and is located on 15q11, 23.1 Mb from the p-telomere. The GABRB3 (9 exons) spans ~230 kb of genomic DNA and is located on 15q13, 24.3 Mb from the p-telomere. The MTHFR (12 exons) spans ~20 kb of genomic DNA and is located on 1p36, 11.8 Mb from the p-telomere. The P336-A1 probemix contains one probe for each exon of UBE3A and two probes for exons 1, 2, 9 and 14. In addition, 3 probes for GABRB3, 4 probes for MTHFR copy number changes and one probe for the MTHFR A222V mutation have been included. This probemix also contains four probes for the 16p13 region, including probes for the AXIN1 gene. Finally, 13 reference probes are included in this probemix, detecting 10 different autosomal chromosomal locations.

No probes are present in P336-A1 for the AS-SRO region. Probes for this region, located within the SNRPN gene, are present in the ME028 probemix. ME028 is the primary MLPA probemix for PWS/Angelman syndrome, detecting both copy number changes and imprinting defects of the 15q11 region. This P336 UBE3A MLPA probemix may be useful for AS cases where no defect was apparent when using the ME028 probemix.

This SALSA® MLPA® probemix is designed to detect deletions/duplications of one or more sequences in the aforementioned gene(s) and to detect the presence of the aforementioned mentioned point mutation in a DNA sample. Heterozygous deletions of recognition sequences should give a 35-50% reduced relative peak area of the amplification product of that probe. Note that a mutation or polymorphism in the sequence detected by a probe can also cause a reduction in relative peak area, even when not located exactly on the ligation site! In addition, some probe signals are more sensitive to sample purity and small changes in experimental conditions. Therefore, deletions and duplications detected by MLPA should always be confirmed by other methods. Not all deletions and duplications detected by MLPA will be pathogenic; users should always verify the latest scientific literature when interpreting their findings. We have no information on what percentage of defects in these genes is caused by deletions/duplications of complete exons. Finally, note that most defects in this gene are expected to be small (point) mutations which will not be detected by this SALSA® MLPA® test.

SALSA® MLPA® probemixes and reagents are sold by MRC-Holland for research purposes and to demonstrate the possibilities of the MLPA technique. They are not CE/FDA certified for use in diagnostic procedures. Purchase of the SALSA® MLPA® test probemixes and reagents includes a limited license to use these products for research purposes.

The use of a SALSA® MLPA® probemix and reagents requires a thermocycler with heated lid and sequence type electrophoresis equipment. Different fluorescent PCR primers are available. The MLPA technique has been first described in Nucleic Acid Research 30, e57 (2002).

Related SALSA® MLPA® probemixes

- P343 Autism Contains additional probes for the 15q13 region and the UBE3A gene
- ME028 PWS-AS: Primary screening for Prader Willi and Angelman Syndrome (copy number & methylation)

More information

Website : www.mlpa.com
E-mail : info@mlpa.com (information & technical questions); order@mlpa.com (for orders)
Mail : MRC-Holland bv; Willem Schoutenstraat 6, 1057 DN Amsterdam, the Netherlands

Data analysis

The P336-A1 UBE3A probemix contains 43 MLPA probes with amplification products between 129 and 454 nt. Included is one mutation-specific probe for the MTHFR A222V mutation, which will only generate a signal when the mutation is present. In addition, it contains 9 control fragments generating an amplification product smaller than 120 nt: four DNA Quantity fragments (Q-fragments) at 64-70-76-82 nt, three DNA denaturation control fragments (D-fragments) at 88-92-96 nt, one X-fragment at 100 nt and one Y-fragment at 105 nt. More information on how to interpret observations on these control fragments can be found in the MLPA protocol.

Data generated by this probemix can first be normalised intra-sample by dividing the peak area of each probe's amplification product by the total area of only the reference probes in this probemix (block normalisation). Secondly, inter-sample normalisation can be achieved by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples. Please note that this type of normalisation assumes no changes occurred in the genomic regions recognised by the reference probes.

Data normalisation should be performed within one experiment. Only samples purified by the same method should be compared. Confirmation of most exons deletions and amplifications can be done by e.g. Southern blotting, long range PCR, qPCR, FISH.

Note that Coffalyser, the MLPA analysis tool developed at MRC-Holland, can be downloaded free of charge from our website www.mlpa.com.

Many copy number alterations in healthy individuals are described in the database of genomic variants: http://projects.tcag.ca/variation. For example, a duplication of a complete gene might not be pathogenic, while a partial duplication or a deletion may result in disease. For some genes, certain in-frame deletions may result in a very mild, or no disease. Copy number changes of reference probes are unlikely to be the cause of the condition tested for. Users should always verify the latest scientific literature when interpreting their findings.

This probemix was developed by R. Vijzelaar at MRC-Holland. In case the results obtained with this probemix lead to a scientific publication, it would be very much appreciated if the probemix designer could be made a co-author.

Info/remarks/suggestions for improvement: info@mlpa.com.

Table 1. SALSA MLPA P336-A1 UBE3A probemix

Length (nt)	SALSA MLPA probe	Chromosomal position Reference / UBE3A / GABRB3 / MTHFR / 16p13.3
64-70-76-82	Q-fragments: DNA quantity; only visible with less than 100 ng sample DNA	
88-92-96	D-fragments: Low signal of 88 or 96 nt fragment indicates incomplete denaturation	
100	X-fragment: Specific for the X chromosome	
105	Y-fragment: Specific for the Y chromosome	
129	Reference probe 11622-L12379	10q25
138	Reference probe 06026-L07190	11p13
142	**UBE3A probe** 10883-L11553	**Exon 9**
148	**MTHFR probe** 12083-L12971	**Exon 5**
155	Reference probe 07144-L06756	19q13
163	Reference probe 10418-L10970	9q34
172 ±	**MTHFR probe** 12084-L12972	**A222V mut**
179	**UBE3A probe** 10882-L11552	**Exon 9**
184	**GABRB3 probe** 10868-L11538	**Exon 7**
191 ∞	AXIN1 probe 10188-L16066	16p13.3
197	**UBE3A probe** 13727-L15208	**Exon 2**
203	**GABRB3 probe** 10873-L11543	**Exon 10**
208	Reference probe 09865-L08705	13q32
215	**UBE3A probe** 10885-L11555	**Exon 11**
220	**MTHFR probe** 12085-L14676	**Exon 10**
226	Reference probe 13598-L15056	9q21
232	**UBE3A probe** 13728-L15209	**Exon 7**
238	AXIN1 probe 10189-L13393	16p13.3
244	**UBE3A probe** 10886-L14677	**Exon 12**
250	**GABRB3 probe** 10866-L11536	**Exon 3**
258	**UBE3A probe** 01317-L14678	**Exon 13**
265	**MTHFR probe** 12086-L13395	**Exon 3**
272	CREBBP probe 09897-L10310	16p13.3
283	**UBE3A probe** 10879-L11549	**Exon 5b**
292	**UBE3A probe** 13729-L15210	**Exon 1**
301	Reference probe 09031-L09285	2q37
310	**UBE3A probe** 10884-L11554	**Exon 10**
319	**MTHFR probe** 12087-L13396	**Exon 8**
328	Reference probe 08543-L08544	3q24
341	**UBE3A probe** 14083-L15682	**Exon 14**
349	Reference probe 13442-L14897	17p13
359 Ж	**UBE3A probe** 13731-SP0136-L15212	**Exon 8**
365	**UBE3A probe** 13732-L16039	**Exon 3**
372	**UBE3A probe** 14012-L15214	**Exon 6**
379	Reference probe 09142-L09953	10q26
390	**UBE3A probe** 14490-L16067	**Exon 4**
400	Reference probe 07678-L06854	7p14
409	**UBE3A probe** 13735-L15216	**Exon 2**
418	**UBE3A probe** 14489-L16371	**Exon 1**
427	Reference probe 08046-L07827	5p15
436	**UBE3A probe** 14085-L15684	**Exon 14**
445	TSC2 probe 02445-L01409	16p13.3
454	Reference probe 08579-L08580	17q23

Ж This probe consists of three parts and has two ligation sites.
∞ This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.
± Mutation-specific probe. This probe will only generate a signal when the A222V mutation is present. It has been tested on artificial test DNA **but not on positive human samples!**

Note: The GABRB3 exon numbering has changed. From description version 02 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for the GABRB3 gene. The exon numbering used here may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2. Complete probe sequences are available on request: info@mlpa.com.

Table 2. P336 probes arranged according to chromosomal location

Table 2a. 15q11 region

Length (nt)	SALSA MLPA probe	Gene Exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		GABRB3	**NM_021912.4**		
		start codon	*65-67 (ex 1)*		
250	10866-L11536	Exon 3	226-227	CGCCTAAGACCC-GACTTCGGGGGT	151.2 kb
184	10868-L11538	Exon 7 (5)	376-377	GGGATCCCTCTC-AACCTCACGCTT	53.7 kb
203	10873-L11543	Exon 10 (7)	40 nt before exon 10 (7)	TTAGTCTGCCAT-GTTGTTTCTCCA	1128.8 kb
		stop codon	*1484-1486 (ex 12)*		
		UBE3A	**NM_130839.1**		
		start codon	*658-660 (ex 3)*		
292	13729-L15210	Exon 1	57-58	GGCCTTTTCCCT-TCGCCAGGACCC	0.4 kb
418	14489-L16371	Exon 1	4 nt after exon 1	GACGACAGGTCA-GTGTTGCCGCGG	26.6 kb
197	13727-L15208	Exon 2	549-550	CAGAAGTTTGGC-GAAATATGGTAT	0.1 kb
409	13735-L15216	Exon 2	134 nt after exon 2	CTAACTACACTT-CCAAGACTGTAT	2.7 kb
365	13732-L16039	Exon 3	659-660	ATGTCACCGAAT-GGCCACAGCTTG	0.5 kb
390	14490-L16067	Exon 4	NM_000462.2; 788-789	CAGGATGGAGAA-GCTGCACCAGTG	3.3 kb
283	10879-L11549	Exon 5b	131 nt after exon 5b	ATGGGAGATAGG-AACATACCTACT	29.8 kb
372	14012-L15214	Exon 6	925-926	AGAAAGGAGCAA-GCTCAGCTTACC	4.6 kb
232	13728-L15209	Exon 7	1830-1831	AATCACAATGAA-GAAGATGATGAA	10.6 kb
359 Ж	13731-SP0136-L15212	Exon 8	2337-2338; 2364-2365	GAAGGAGAACAA- 27nt spanning oligo -GAATTTTTTCAG	3.6 kb
179	10882-L11552	Exon 9	2480-2481	AACTGAGGGTCA-GTTTACTCTGAT	0.1 kb
142	10883-L11553	Exon 9	2569-2570	TCTACAGGAAGC-TAATGGGGAAAA	0.8 kb
310	10884-L11554	Exon 10	2692-2693	TCACTTTCCAGA-TATCACAGACAG	1.4 kb
215	10885-L11555	Exon 11	2869-2870	TTCATATGGTGA-CCAATGAATCTC	0.2 kb
244	10886-L14677	Exon 12	3009-3010	TCTGTTCTGATT-AGGTGAGGTACT	14.2 kb
258	01317-L14678	Exon 13	3041-3042	TCATTCATTTAC-AGATGAACAGAA	1.8 kb
341	14083-L15682	Exon 14	4021-4022	GTCTTGCAATGA-ACTGTTTCAGTA	0.4 kb
436	14085-L15684	Exon 14	4390-4391	TACTTAATCATA-CAGTAAGCTGAC	
		stop codon	*3274-3276 (ex 14)*		

Ж This probe consists of three parts and has two ligation sites.

The NM_021912.4 sequence represents transcript variant 2 and is a reference standard in the NCBI RefSeqGene project. The NM_130839.1 sequence represents transcript variant 3.

Note: The GABRB3 exon numbering has changed. From description version 02 onwards, we have adopted the NCBI exon numbering that is present in the NM_ sequences for the GABRB3 gene. The exon numbering used here may differ from literature! The exon numbering used in previous versions of this product description can be found between brackets in Table 2. Complete probe sequences are available on request: info@mlpa.com.

Table 2b. AXIN1 gene / 16p13.3

Length (nt)	SALSA MLPA probe	AXIN1 Exon	Ligation site NM_003502.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*178-180 (ex 2)*		
238	10189-L13393	Exon 11	2959-2960	GATCATCGGCAA-AGTGGAGAAGGT	58.5 kb
191 ∞	10188-L16066	Exon 2	759-760	CCTGCACTGGCT-TCAGGAAGCTGG	1741.9 kb
		stop codon	*2764-2766 (ex 11)*		
445	02445-L01409	TSC2 Exon 41		ACACCTGGCTAT-GAGGTGGGCCAG	1669.4 kb
272	09897-L10310	CREBBP Exon 18		CTGGCTCATGTT-CAACAATGCCTG	

∞ This probe is located within, or close to, a very strong CpG island. A low signal of this probe can be due to incomplete sample DNA denaturation, e.g. due to the presence of salt in the sample DNA.

The NM_003502.2 sequence represents transcript variant 1 and is a reference standard in the NCBI RefSeqGene project.

Table 2c. MTHFR gene

Length (nt)	SALSA MLPA probe	MTHFR Exon	Ligation site NM_005957.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*230-232 (ex 2)*		
265	12086-L13395	Exon 3	15nt before exon 3	CTCTCTTCAGAA-ACAAACCCCCTA	5.1 kb
172 ±	12084-L12972	Exon 5	894-893 reverse	TGATGAAATCGA-CTCCCGCAGACA	0.1 kb
148	12083-L12971	Exon 5	972-973	CGACATGGGCAT-CACTTGCCCCAT	1.8 kb
319	12087-L13396	Exon 8	1452-1453	CCTCTTCTACCT-GAAGAGCAAGTC	2.1 kb
220	12085-L14676	Exon 10	1773-1774	CTACTTAGAGTT-TTTCACTTCCCG	
		stop codon	*2198-2200 (ex 12)*		

± Mutation-specific probe. This probe will only generate a signal when the A222V mutation is present. It has been tested on artificial test DNA **but not on positive human samples!**

The NM_005957.4 sequence is a reference standard in the NCBI RefSeqGene project.

Note: Exon numbering might be different as compared to literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

SALSA MLPA probemix P336-A1 UBE3A sample picture

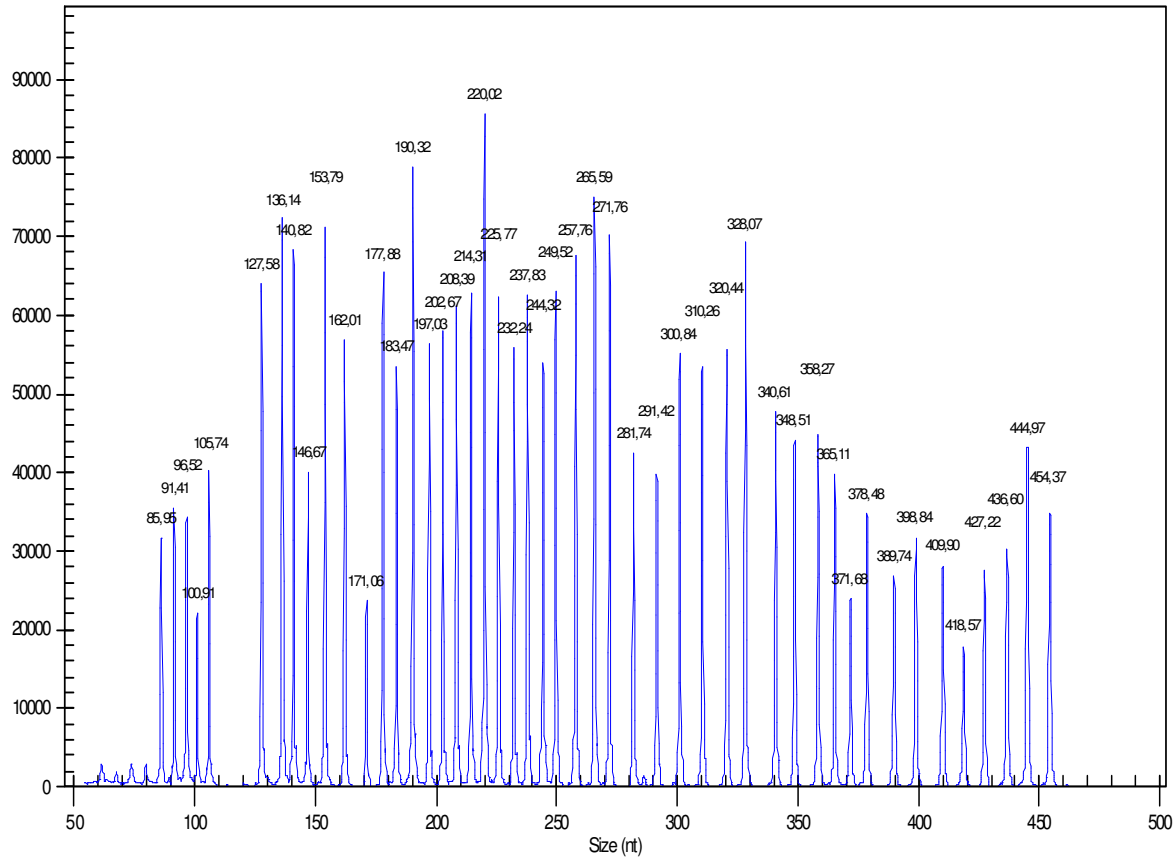

Figure 1. Capillary electrophoresis pattern from a sample of approximately 50 ng human male control DNA analysed with SALSA® MLPA® probemix P336-A1 UBE3A (lot A1-0909).

Implemented Changes – compared to the previous product description version(s).
Version 02 (48) - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Tables have been numbered. - Data analysis method has been modified. - Exon numbering of the GABRB3 gene has been changed on page 3 and 4. - Various minor textual changes on page 1. - Remark on RefSeqGene standard and transcript variant added below Table 2. - Ligation sites of the probes targeting the GABRB3, AXIN1 and UBE3A genes updated according to new version of the NM_reference sequence. *Version 01 (44)* - Not applicable, new document.