Product Description SALSA® MLPA® Probemix P297-D1 Microdeletion Syndromes-2

To be used with the MLPA General Protocol.

Version D1. As compared to version C1, the probemix has been largely redesigned. All probes targeting regions 7q36.1, 12p11.23, 18q21.2 and 20p12.2 have been removed. All other regions have been thoroughly revised and their coverage was improved. For complete product history see page 9.

Catalogue numbers:
- **P297-025R:** SALSA MLPA Probemix P297 Microdeletion Syndromes-2, 25 reactions.
- **P297-050R:** SALSA MLPA Probemix P297 Microdeletion Syndromes-2, 50 reactions.
- **P297-100R:** SALSA MLPA Probemix P297 Microdeletion Syndromes-2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information: The SALSA MLPA Probemix P297 Microdeletion Syndromes-2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the following chromosomal regions:

• 1q21.1 (TAR)	5 probes
• 1q21.1 (distal)	6 probes
• 3q29	4 probes
• 15q13	10 probes
• 15q24	4 probes
• 16p13.11	4 probes
• 16p12.1	3 probes
• 16p12.1-p11.2	3 probes
• 16p11.2 (distal)	3 probes
• 16p11.2 (proximal)	4 probes
• 17q12	8 probes

More information about these Microdeletion Syndromes is available below Table 2.

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 MLPA Probemix P297-D1 Microdeletion Syndromes-2 contains 54 MLPA probes with amplification products between 115 and 519 nucleotides (nt).

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 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.mrcholland.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 developmental delay and/or intellectual disability syndromes. 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. Sample ID numbers as indicated in the table below from the Coriell Institute have been tested with this P297-D1 probemix at MRC-Holland and can be used as a positive control samples to detect the aberrations described in the table. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID	Affected P297-D1 region	Affected probes
NA03563	3q29	Duplication 409, 204, 191 and 509 nt probes
NA10175		
NA11428		
NA22976		
NA03184	15q13.1 – 15q24.2	Duplication 400, 211, 197, 519, 166, 244, 232, 125, 292, 424, 500, 362, 132 and 355 nt probes
NA13685	16p13.11	Deletion 458, 147 and 329 nt probes
NA06226	16p13.11 – 16p12.1	Duplication 458, 147, 329, 226, 136, 442 and 115 nt probes
NA08039	16p13.11 – 16p12.1	Duplication 458, 147, 329, 226, 136, 442, 115, 337 and 266 nt probes
NA05875	16p12.1 – 16p11.2	Deletion 465, 391, 493, 483, 416, 346, 178 and 302 nt probes

Data analysis: There are no dedicated reference probes but instead all peaks are used for normalisation. Data generated by this probemix can be normalised intra-sample by dividing the peak height of each amplification product by the combined peak height of all peaks in that sample (global 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.

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of each individual probe over all the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the 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. 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.
- 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.

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.

Database of genomic variation and phenotype in humans using Ensembl resources (DECIPHER): https://decipher.sanger.ac.uk/. We strongly encourage users to deposit positive results in the DECIPHER Database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNPs and unusual results (e.g., a duplication of two probes that are not consecutive in location) to MRC-Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P297-D1 Microdeletion Syndromes-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Location (hg18) in kb
64-105	Control fragments – see table in probemix content section for more information		
115 *	**CDR2 probe** S1229-L31953	**16**p12.1	16-022.267
121 *	**ZNHIT3 probe** S1230-L32054	**17**q12	17-031.924
125	**CHRNA7 probe** S1140-L29533	**15**q13.3	15-030.181
132 *	**EDC3 probe** 22700-L32072	**15**q24.1	15-072.754
136 *	**UQCRC2 probe** 20583-L28241	**16**p12.1	16-021.876
142 ¥	**CD160 probe** 22687-L31938	**1**q21.1	01-144.415
147 *	**MYH11 probe** 22701-L31956	**16**p13.11	16-015.840
152 ¥	**AATF probe** 21021-L32055	**17**q12	17-032.463
160	**HNF1B probe** 07699-L12885	**17**q12	17-033.174
166	**KLF13 probe** 08376-L08230	**15**q13.3	15-029.452
172 ¥	**HNF1B probe** 22688-L31939	**17**q12	17-033.168
178	**HIRIP3 probe** 11667-L14462	**16**p11.2	16-029.914
185 *	**NUDT17 probe** 22702-L31957	**1**q21.1	01-144.299
191 *	**PAK2 probe** 22703-L31958	**3**q29	03-197.994
197 *	**FAN1 probe** 22704-L31959	**15**q13.3	15-029.000
204 * «	**FBXO45 probe** 22705-L31960	**3**q29	03-197.789
211 * #	**ARHGAP11B probe** 22707-L31962	**15**q13.2	15-028.706
220	**FMO5 probe** 12944-L14099	**1**q21.1	01-145.125
226 *	**XYLT1 probe** 22708-L31963	**16**p12.3	16-017.260
232 * «	**OTUD7A probe** 22709-L31964	**15**q13.3	15-029.950
238 *	**HNF1B probe** 16906-L19835	**17**q12	17-033.145
244 *	**OTUD7A probe** 22710-L31965	**15**q13.3	15-029.607
250 ¥	**GJA5 probe** 22689-L31940	**1**q21.1	01-145.697
256 ¥	**BCL9 probe** 12945-L31941	**1**q21.1	01-145.563
266 *	**LCMT1 probe** 22711-L31966	**16**p12.1	16-025.051
274 ¥	**HFE2 (HJV) probe** 22690-L31942	**1**q21.1	01-144.128
283 ¥	**GJA8 probe** 22691-L31943	**1**q21.1	01-145.848
292 ¥	**CHRNA7 probe** 22692-L31944	**15**q13.3	15-030.191
302	**MAPK3 probe** 11670-L14454	**16**p11.2	16-030.041
310 * «	**ACACA probe** 22712-L31967	**17**q12	17-032.840
319	**PRKAB2 probe** 12949-L14104	**1**q21.1	01-145.097
329 *	**ABCC6 probe** 22693-L31945	**16**p13.11	16-016.163
337	**PALB2 probe** 07504-L07166	**16**p12.1	16-023.522
346 *	**MVP probe** 22713-L31968	**16**p11.2	16-029.753
355 *	**SIN3A probe** 22714-L31969	**15**q24.2	15-073.510
362 *	**STRA6 probe** 22715-L31970	**15**q24.1	15-072.272

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Location (hg18) in kb
370 ¥	**LHX1 probe** 08396-L31946	**17**q12	17-032.372
382 ¥	**PEX11B probe** 22694-L31947	**1**q21.1	01-144.229
391 *	**ATXN2L probe** 22716-L31971	**16**p11.2	16-028.745
400 ¬	**TJP1 probe** 08399-L14456	**15**q13.1	15-027.784
409 *	**RNF168 probe** 22717-L31972	**3**q29	03-197.700
416 ¥ «	**MAZ probe** 22695-L31948	**16**p11.2	16-029.728
424 ¥ ¬	**SCG5 probe** 12951-L31949	**15**q13.3	15-030.776
433 *	**HNF1B probe** 21371-L29819	**17**q12	17-033.121
442 *	**VWA3A probe** 22718-L31973	**16**p12.1	16-022.016
451 *	**RBM8A probe** 22719-L31974	**1**q21.1	01-144.223
458 *	**MARF1 probe** 22720-L31975	**16**p13.11	16-015.603
465 ¥	**IL21R probe** 22696-L31950	**16**p12.1	16-027.353
475 ¥	**ACP6 probe** 22697-L31951	**1**q21.1	01-145.609
483 *	**LAT probe** 11677-L12448	**16**p11.2	16-028.905
493 *	**RABEP2 probe** 22721-L31976	**16**p11.2	16-028.834
500 ¥ ¬	**PML probe** 22698-L31952	**15**q24.1	15-072.078
509 ¥	**DLG1 probe** 22699-L29663	**3**q29	03-198.510
519 *	**TRPM1 probe** 22722-L31977	**15**q13.3	15-029.150

* New in version D1.

¥ Changed in version D1. Minor alteration, no change in sequence detected.

« 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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking probes are unlikely to be related to the condition tested.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Table 2. P297-D1 probes arranged according to chromosomal location

Table 2a. 1q21.1

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
TAR – 200-kb minimally deleted region				
274	22690-L31942	HFE2 (HJV)	TCCAAGCTGCCT-ACATTGGCACAA	95.2 kb
451	22719-L31974	RBM8A	CGTAACTCCAAA-CAGTTCACAAAA	5.7 kb
382	22694-L31947	PEX11B	TGAGTTACAGAA-ACAGATTCGACA	70.4 kb
185	22702-L31957	NUDT17	GTGATGGTATTT-GGGTAAACCCCA	116.3 kb
TAR – 500-kb deletion				
142	22687-L31938	CD160	CCATAAGCCAAG-TCACACCGTTGC	681.7 kb
Distal 1q21.1 Recurrent Microdeletion				
319	12949-L14104	PRKAB2	AACACAAAACTT-ATTGGGTAAGTG	28.3 kb
220	12944-L14099	FMO5	AACGCCATACCA-TTCAGGGAGACT	437.4 kb
256	12945-L31941	BCL9	TTATTCCATCTG-AGAAGCCCAGCC	45.8 kb
475	22697-L31951	ACP6	CGACCGCAGCCT-GCTGAAGTTGAA	88.8 kb
250	22689-L31940	GJA5	GTACTTCATCTA-CGGAATCTTCCT	150.5 kb
283	22691-L31943	GJA8	CGGTTAGATCGT-CTGACCTGGCTC	

- Thrombocytopenia Absent Radius (TAR) syndrome is characterised by a reduction in the number of platelets and bilateral absence of the radius in the presence of both thumbs.
- As described by Klopocki et al., the minimally deleted segment is a 200-kb region, that encompasses at least 10 genes (*HFE2* (*HJV*) – *NUDT17*), with *RBM8A* as the critical gene. The most frequently observed deleted allele is a 500-kb deletion that spans an additional five genes (*HFE2* (*HJV*) – *GPR89*).
- For more information on TAR syndrome see https://www.ncbi.nlm.nih.gov/books/NBK23758/ and https://www.omim.org/entry/274000.
- The distal 1q21.1 Recurrent Microdeletion of 1.35-Mb does not have obvious clinical findings. The following characteristics can be suggestive of this 1q21.1 Recurrent Microdeletion: developmental delays, mild-to-moderate intellectual disability, mild dysmorphic facial features and microcephaly. The clinical significance of this common microdeletion syndrome is uncertain.
- Although several genes of interest are within the distal 1.35-Mb deletion, no single gene in which pathogenic variants are causative has been identified.
- Although less frequent, individuals with 1q21.1 Duplication Syndrome have also been reported (see https://www.omim.org/entry/612475). Some of the phenotypic features may include: hypotonia, macrocephaly, a prominent forehead and developmental delay.
- For more information on the 1q21.1 Recurrent Microdeletion see https://www.ncbi.nlm.nih.gov/books/NBK52787/ and https://www.omim.org/entry/612474.

Table 2b. 3q29

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
409	22717-L31972	RNF168	AACCTGGGGAAC-TGAGAAGAGAAT	88.9 kb
204 «	22705-L31960	FBXO45	ACTTTACATCGA-AACCCCATTGCT	205.3 kb
191	22703-L31958	PAK2	TTCTCAGGCACA-GAGAAAGGTAAA	515.7 kb
509	22699-L29663	DLG1	TGGATCTGGTGT-AGGCGAGGTCAC	

- The 3q29 Recurrent Deletion is characterised by global developmental delay and/or intellectual disability, speech delay and increased risk for neuropsychiatric disorders.
- The Recurrent Deletion is approximately 1.6-kb and includes several genes of interest. No single gene in which pathogenic variants are causative has been identified.
- A few individuals with 3q29 Duplication Syndrome have been reported (see https://omim.org/entry/611936).
- For more information on the 3q29 Recurrent Deletion see https://www.ncbi.nlm.nih.gov/books/NBK385289/ and https://omim.org/entry/609425.

Table 2c. 15q13

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
400 ¬	08399-L14456	TJP1	CCTTTGGTGATG-TGTGGTCCCCAT	922.7 kb
211 #	22707-L31962	ARHGAP11B	AGCACGATCTGC-TAATAAGTGATG	293.6 kb
197	22704-L31959	FAN1	TACTGCAGAGAC-TTCACATGTATG	149.4 kb
519	22722-L31977	TRPM1	CAAGCACACCCA-GAGCTACCCAAC	302.5 kb

166	08376-L08230	KLF13	TTGAACCCCCTT-TCTCAGGGATGG	154.7 kb
244	22710-L31965	OTUD7A	CGGAAAGCTCTC-TATACCATGATG	343.3 kb
232 «	22709-L31964	OTUD7A	GCCGCTACCCGA-CTTCCATTTTCT	230.8 kb
125	S1140-L29533	CHRNA7	TGCAAATGGTAA-GTTAAGAGAATG	10.5 kb
292	22692-L31944	CHRNA7	AGACTGTTCGTT-TCCCAGATGGCC	584.8 kb
424 ¬	12951-L31949	SCG5	TCAGCATGGCTT-ATGTGCACGTGT	

- Individuals with the 15q13.3 Microdeletion Syndrome are at increased risk for a wide range of clinical manifestations including intellectual disability, seizures, autism spectrum disorders and schizophrenia. A subset of persons with the deletion have no obvious clinical findings.
- The 15q13.3 Microdeletion is a recurrent 2.0-Mb deletion, of which 1.5-Mb is unique sequence and 500-kb consists of segmental duplications. Specific genes implicated in the phenotype include *CHRNA7* and *OTUD7A*, both of which reside in the critical region. Individuals with larger (~4-Mb) or smaller (<700-kb) have been described. These smaller deletions overlap *CHRNA7* only or *CHRNA7* and the first exon of *OTUD7A* (the latter is targeted by the 232 nt probe).
- Duplication of this region has also been described, see https://omim.org/entry/608636.
- For more information on the 15q13.3 Microdeletion see https://www.ncbi.nlm.nih.gov/books/NBK50780/ and https://omim.org/entry/612001.

Table 2d. 15q24

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
500 ¬	22698-L31952	PML	CGGTGCGTGAGT-TCCTGGACGGCA	192.8 kb
362	22715-L31970	STRA6	GACACTGCATCT-ACACTCCACAGC	483.8 kb
132	22700-L32072	EDC3	GGCCTTTCCATA-ATGGAGTGAAGT	755.2 kb
355	22714-L31969	SIN3A	GAGACCATGCAG-TCAGCTACGGGA	

- The 15q24 Microdeletion Syndrome, also known as Witteveen-Kolk syndrome (WITKOS) is characterised by global developmental delay, mild to severe intellectual disability, facial dysmorphisms, congenital malformations and growth retardation.
- The majority of 15q24 deletions identified involve a 1.1-Mb critical region. There is evidence suggesting that *SIN3A* is the critical gene in WITKOS.
- For more information on the Witteveen-Kolk syndrome see https://www.ncbi.nlm.nih.gov/books/NBK84258/ and https://omim.org/entry/613406.

Table 2e. 16p

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
			16p13.11	
458	22720-L31975	MARF1	TTGCAAAGAATG-TGCGGTCTTTAC	236.1 kb
147	22701-L31956	MYH11	AAGGGCCAACTC-AGTGACGATGAG	323.3 kb
329	22693-L31945	ABCC6	GTGCTGAGCAAA-GCCCACCTCAGT	1097.6 kb
226	22708-L31963	XYLT1	CCCTAAGTGTGA-CATCTCAGGCAA	4615.9 kb
			16p12.1	
136	20583-L28241	UQCRC2	TTTCCAAACACT-TGAGGAAGTGAA	140.1 kb
442	22718-L31973	VWA3A	TCGGACAATGGA-TTATTGGTTACA	250.1 kb
115	S1229-L31953	CDR2	CTTGCAAGGTCA-GCCAAGCCCTGA	1255.9 kb
			16p12.1-p11.2	
337	07504-L07166	PALB2	TGTGCAGCAGCA-ATCTTGACTTCT	1528.8 kb
266	22711-L31966	LCMT1	GTCTTCCGTAGA-AATGCCTTTATA	2301.9 kb
465	22696-L31950	IL21R	TGCTACACCGAT-TACCTCCAGACG	1392.0 kb
			16p11.2 (distal)	
391	22716-L31971	ATXN2L	TAGTAGCATAGT-TGAAGTCAACAT	88.4 kb
493	22721-L31976	RABEP2	GAAGCTGCGGGA-GATCGTACTGCC	71.1 kb
483	11677-L12448	LAT	ACCAGTTTGTAT-CCAAGGGGCATC	823.7 kb
			16p11.2 (proximal)	
416 «	22695-L31948	MAZ	CCACGGCAGCAT-ACCTGCGCATCC	24.2 kb
346	22713-L31968	MVP	CCCTCCATCTAA-AGGCGCTGCTTG	161.4 kb
178	11667-L14462	HIRIP3	CCAGGGAAGACA-AACTGGACCTTA	126.8 kb
302	11670-L14454	MAPK3	ACGTGCGCAAGA-CTCGCGTGGCCA	

The pericentric region of chromosome 16, specifically involving 16p12-p11, is a structurally complex region enriched in repetitive sequence elements, rendering this region susceptible to deletion or rearrangement. There are several phenotypes associated with variation in this region.

- The 16p13.11 Recurrent Deletion/Duplication has been described by Redaelli et al. The shared regions span over 3.2-Mb, while the smallest region of overlap (SRO) is 687-kb. The SRO encompasses four OMIM genes: *MARF1*, *NDE1*, *MYH11* and *FOPNL*. Additionally, *ABCC1* is partially included in the SRO. A proximal larger region that includes *ABCC1*, *ABCC6*, *NOMO3* and *XYLT1* was shared by four out of seven cases.
- The 16p12.1 Recurrent Deletion (520-kb) is associated with susceptibility to childhood developmental delay or intellectual disability, including schizophrenia. For more information see https://www.ncbi.nlm.nih.gov/books/NBK274565/ and https://omim.org/entry/136570.
- The 16p12.1-p11.2 Recurrent deletion (7.1- to 8.7-Mb) is characterised by dysmorphic facial features, feeding difficulties, recurrent ear infections, developmental delay and cognitive impairment. For more information see https://omim.org/entry/613604.
- The 16p11.2 region contains two adjacent Recurrent Microdeletions. The distal Recurrent Microdeletion/Duplication typically spans a 220-kb region, encompassing approximately nine OMIM genes including *ATXN2L*, *RABEP2* and *LAT*. The proximal Recurrent Microdeletion/Duplication typically spans a 593-kb region, encompassing (amongst others) the following OMIM genes: *MAZ*, *MVP*, *HIRIP3* and *MAPK3*. For more information on the distal region see https://omim.org/entry/613444. More information on the proximal region can be found on https://www.ncbi.nlm.nih.gov/books/NBK11167/ and https://omim.org/entry/611913.

Table 2f. 17q12

Length (nt)	SALSA MLPA probe	Gene	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
121	S1230-L32054	ZNHIT3	TCTTCCTCATCA-CTATTGAGAAAA	447.9 kb
370	08396-L31946	LHX1	TAGCGACCTGGT-GCGGAGAGCGCG	91.3 kb
152	21021-L32055	AATF	CAAGCTACTGAG-TTTCATGGCACC	377.4 kb
310 «	22712-L31967	ACACA	AGAGGATGTGGT-GGTCTACTCTGA	281.0 kb
433	21371-L29819	HNF1B	GCCTGGTGATGC-CCACACACCACT	23.1 kb
238	16906-L19835	HNF1B	CTCCAGAGCGAC-AATGGCCCAGGT	23.3 kb
172	22688-L31939	HNF1B	AGACAAAAGCAG-TCAGGATCAGCT	5.8 kb
160	07699-L12885	HNF1B	TGCAGCAACACA-ACATCCCCCAGA	

- The 17q12 Recurrent Deletion of 1.4-Mb is characterised by variable combinations of the three following findings: structural or functional abnormalities of the kidney and urinary tract, maturity-onset diabetes and neurodevelopmental or neuropsychiatric disorders.
- A duplication of the same region has also been described: https://omim.org/entry/614526.
- For more information see https://www.ncbi.nlm.nih.gov/books/NBK401562/ and https://omim.org/entry/614527.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking probes are unlikely to be related to the condition tested.
This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
« 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) Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Related SALSA MLPA probemixes

- P245 Microdeletion Syndromes 1A: probes are included for 23 different microdeletion syndromes.
- P064 Microdeletion Syndromes 1B: contains probes for 1p36, Wolf-Hirschhorn, Cri du Chat, Sotos, Saethre-Chotzen, Williams-Beuren, Langer-Giedion, WAGR, Prader-Willi/Angelman, Rubinstein-Taybi, Miller-Dieker, Smith-Magenis, Alagille, DiGeorge and Phelan-McDermid syndrome.
- P106 MRX: X-linked mental retardation.
- P036/P070 Subtelomeres: these probemixes contain one probe for every subtelomere.
- More probemixes for specific microdeletion syndromes, e.g. Rett, DiGeorge, Prader-Willi, Lissencephaly, Canavan and Williams-Beuren syndrome, are available. See www.mrcholland.com.

References

- Klopocki E., et al. (2007). Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. Am J Hum Genet. 80:232-40.
- Redaelli S., et al. (2019). Refining the Phenotype of Recurrent Rearrangements of Chromosome 16. *Int J Mol Sci.* 20:1095.
- 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.
- 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.

P297 Product history	
Version	*Modification*
D1	The probemix has been largely redesigned. All probes targeting regions 7q36.1, 12p11.23, 18q21.2 and 20p12.2 have been removed. All other regions have been thoroughly revised and their coverage was improved.
C1	One extra CHRNA7 target probe has been included.
B2	The 88 and 96 nt DNA denaturation fragments have been replaced.
B1	The probes for the 2p16.1 microdeletion syndrome have been removed and several new microdeletion syndromes probes have been included.
A1	First release.

Implemented changes in the product description

Version D1-01 — 06 February 2020 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Warning added to Table 2c for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 13 – 20 November 2018 (55)
- Additional information about two target locations for PDE4DIP (probe 05712-L05712 at 130 nt) added to Table 1 and Table 2.
- Minor textual changes.

Version 12 – 30 August 2016 (55)
- Product description adapted to a new version (version and lot number changed, new picture included).
- Minor textual changes in remarks of Table 2a – 2j and addition of separate references chapter.
- Updated description of related products.

Version 11 – 04 November 2015 (55)
- Product description adapted to a new lot (lot number changed, new picture included).
- Minor textual changes.

Version 10 – 07 August 2015 (53)
- Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed.

Version 09 (53)
- Probe name changed in Table 1 & Table 2d and a corresponding note added below these tables.
- Corrected text on data analysis.
- Updated link for "Database of Genomic Variants"

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