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

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 10.

Catalogue numbers:
- **P297-025R:** SALSA MLPA Probemix P297 Microdeletion-2, 25 reactions.
- **P297-050R:** SALSA MLPA Probemix P297 Microdeletion-2, 50 reactions.
- **P297-100R:** SALSA MLPA Probemix P297 Microdeletion-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-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
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive (MANE) database: http://tark.ensembl.org/

Probemix content
The SALSA MLPA Probemix P297-D1 Microdeletion-2 contains 54 MLPA probes with amplification products between 115 and 519 nucleotides (nt). This includes 54 probes for the chromosomal regions 1q21.1, 3q29,

15q13, 15q24, 16p13.11, 16p12.1-p11.2, and 17q12. Complete probe sequences 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 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 different 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 (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. Sample ID numbers NA03563, NA10175, NA11428, NA22976, NA03184, NA13685, NA06226, NA08039 and NA05875 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 below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of copy number alteration*	Altered target genes in P297-D1	Expected copy number alteration
NA03563	Coriell Institute	3q29	*RNF168, FBXO45, PAK2 and DLG1*	Heterozygous duplication
NA10175	Coriell Institute			

NA11428	Coriell Institute			
NA22976	Coriell Institute			
NA03184	Coriell Institute	15q13.1 – 15q24.2	*TJP1, ARHGAP11B, FAN1, TRPM1, KLF13, OTUD7A, CHRNA7, SCG5, PML, STRA6, EDC3 and SIN3A*	Heterozygous duplication
NA13685	Coriell Institute	16p13.11	*MARF1, MYH11 and ABCC6*	Heterozygous deletion
NA06226	Coriell Institute	16p13.11 – 16p12.1	*MARF1, MYH11, ABCC6, XYLT1, UQCRC2, VWA3A and CDR2*	Heterozygous duplication
NA08039	Coriell Institute	16p13.11 – 16p12.1	*MARF1, MYH11, ABCC6, XYLT1, UQCRC2, VWA3A, CDR2, PALB2 and LCMT1*	Heterozygous duplication
NA05875	Coriell Institute	16p12.1 – 16p11.2	*IL21R, ATXN2L, RABEP2, LAT, MAZ, MVP, HIRIP3 and MAPK3*	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P297-D1 probemix.

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.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 final ratio (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	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	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 the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software 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. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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.
- 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.

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 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) mutation database

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 copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P297-D1 Microdeletion-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	16-022,267
121 *	**ZNHIT3 probe** S1230-L32054	**17**q12	17-031,924
125	**CHRNA7 probe** S1140-L29533	**15**q13	15-030,181
132 *	**EDC3 probe** 22700-L32072	**15**q24	15-072,754
136 *	**UQCRC2 probe** 20583-L28241	**16**p12	16-021,876
142 ¥	**CD160 probe** 22687-L31938	**1**q21	01-144,415
147 *	**MYH11 probe** 22701-L31956	**16**p13	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	15-029,452
172 ¥	**HNF1B probe** 22688-L31939	**17**q12	17-033,168
178	**HIRIP3 probe** 11667-L14462	**16**p11	16-029,914
185 *	**NUDT17 probe** 22702-L31957	**1**q21	01-144,299
191 *	**PAK2 probe** 22703-L31958	**3**q29	03-197,994
197 *	**FAN1 probe** 22704-L31959	**15**q13	15-029,000
204 * «	**FBXO45 probe** 22705-L31960	**3**q29	03-197,789
211 *	**ARHGAP11B probe** 22707-L31962	**15**q13	15-028,706
220	**FMO5 probe** 12944-L14099	**1**q21	01-145,125
226 *	**XYLT1 probe** 22708-L31963	**16**p12	16-017,260
232 * «	**OTUD7A probe** 22709-L31964	**15**q13	15-029,950
238 *	**HNF1B probe** 16906-L19835	**17**q12	17-033,145
244 *	**OTUD7A probe** 22710-L31965	**15**q13	15-029,607
250 ¥	**GJA5 probe** 22689-L31940	**1**q21	01-145,697
256 ¥	**BCL9 probe** 12945-L31941	**1**q21	01-145,563
266 *	**LCMT1 probe** 22711-L31966	**16**p12	16-025,051
274 ¥	**HFE2 probe** 22690-L31942	**1**q21	01-144,128
283 ¥	**GJA8 probe** 22691-L31943	**1**q21	01-145,848
292 ¥	**CHRNA7 probe** 22692-L31944	**15**q13	15-030,191
302	**MAPK3 probe** 11670-L14454	**16**p11	16-030,041
310 * «	**ACACA probe** 22712-L31967	**17**q12	17-032,840
319	**PRKAB2 probe** 12949-L14104	**1**q21	01-145,097
329 *	**ABCC6 probe** 22693-L31945	**16**p13	16-016,163
337	**PALB2 probe** 07504-L07166	**16**p12	16-023,522
346 *	**MVP probe** 22713-L31968	**16**p11	16-029,753
355 *	**SIN3A probe** 22714-L31969	**15**q24	15-073,510
362 *	**STRA6 probe** 22715-L31970	**15**q24	15-072,271
370 ¥	**LHX1 probe** 08396-L31946	**17**q12	17-032,372
382 ¥	**PEX11B probe** 22694-L31947	**1**q21	01-144,229
391 *	**ATXN2L probe** 22716-L31971	**16**p11	16-028,745
400 ¬	**TJP1 probe** 08399-L14456	**15**q13	15-027,784
409 *	**RNF168 probe** 22717-L31972	**3**q29	03-197,700
416 ¥ «	**MAZ probe** 22695-L31948	**16**p11	16-029,728
424 ¥ ¬	**SCG5 probe** 12951-L31949	**15**q13	15-030,776
433 *	**HNF1B probe** 21371-L29819	**17**q12	17-033,121
442 *	**VWA3A probe** 22718-L31973	**16**p12	16-022,016
451 *	**RBM8A probe** 22719-L31974	**1**q21	01-144,223
458 *	**MARF1 probe** 22720-L31975	**16**p13	16-015,603
465 ¥	**IL21R probe** 22696-L31950	**16**p12	16-027,353
475 ¥	**ACP6 probe** 22697-L31951	**1**q21	01-145,609
483 *	**LAT probe** 11677-L12448	**16**p11	16-028,905

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Location (hg18) in kb
493 *	**RABEP2 probe** 22721-L31976	**16**p11	16-028,834
500 ¥ ¬	**PML probe** 22698-L31952	**15**q24	15-072,078
509 ¥	**DLG1 probe** 22699-L29663	**3**q29	03-198,510
519 *	**TRPM1 probe** 22722-L31977	**15**q13	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 or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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*	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 – 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 – 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://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	1.1 **Mb**
226	22708-L31963	*XYLT1*	CCCTAAGTGTGA-CATCTCAGGCAA	4.6 **Mb**
			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	1.3 **Mb**
			16p12.1-p11.2	
337	07504-L07166	*PALB2*	TGTGCAGCAGCA-ATCTTGACTTCT	1.5 **Mb**
266	22711-L31966	*LCMT1*	GTCTTCCGTAGA-AATGCCTTTATA	2.3 **Mb**
465	22696-L31950	*IL21R*	TGCTACACCGAT-TACCTCCAGACG	1.4 **Mb**
			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.

[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.

« 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 or reference 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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P036/P070 Subtelomeres	These probemixes contain probes for subtelomeric regions.
P064 Microdeletion Syndromes-1B	Contains probes for 1p36 deletion, 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.
P245 Microdeletion Syndromes-1A	Contains probes for several microdeletion/microduplication syndromes and can be used for primary screening of microdeletion/microduplication syndromes.
P106 X-linked ID	Contains probes for various genes involved in X-linked intellectual disability.

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.

Selected publications using SALSA MLPA Probemix P297 Microdeletion-2

- Coll M., et al. (2017). Targeted next-generation sequencing provides novel clues for associated epilepsy and cardiac conduction disorder/SUDEP. *PLoS One*. 12: e0189618.

- Kowalczyk K., et al. (2022). Application of array comparative genomic hybridization (aCGH) for identification of chromosomal aberrations in the recurrent pregnancy loss. *J Assist Reprod Genet*. 39:357-367.

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 D1-02 – 14 December 2023 (04P) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. 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.

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