Product Description
SALSA® MLPA® Probemix P064-C2 Microdeletion Syndromes-1B

To be used with the MLPA General Protocol.

Version C2
For complete product history see page 14.

Catalogue numbers:
- **P064-025R:** SALSA MLPA Probemix P064 Microdeletion Syndromes-1B, 25 reactions.
- **P064-050R:** SALSA MLPA Probemix P064 Microdeletion Syndromes-1B, 50 reactions.
- **P064-100R:** SALSA MLPA Probemix P064 Microdeletion Syndromes-1B, 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.

Intended purpose
The SALSA MLPA Probemix P064 Microdeletion Syndromes-1B is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of a distinct subset of recurrent microdeletions and microduplications (mentioned in the table below) in genomic DNA isolated from human peripheral whole blood, buccal swabs, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or foetal blood specimens. P064 Microdeletion Syndromes-1B is intended to confirm a potential cause for and clinical diagnosis of developmental delay, intellectual disability and/or congenital anomalies.

This probemix has a limited number of probes for each specific chromosomal region and will therefore not detect all possible causes of the syndromes included. Copy number variations (CNVs) detected with the P064 Microdeletion Syndromes-1B probemix must be confirmed by a designated MLPA follow-up probemix or another technique.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, parental evaluation, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1]Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2]To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Syndromes that can be detected by the P064 probemix			
Syndrome	*Genetic locus*	*OMIM*	*Number of probes*
1p36 deletion syndrome	1p36	607872	4
Wolf-Hirschhorn syndrome	4p16.3	194190	4
Cri-du-Chat syndrome	5p15	123450	5
Sotos syndrome	5q35.3	117550	3
Saethre-Chotzen syndrome	7p21.1	101400	2
Williams-Beuren syndrome	7q11.23	194050	3
Williams-Beuren duplication syndrome	7q11.23	609757	
Trichorhinophalangeal syndrome type 2	8q24.11-q24.13	150230	3
WAGR syndrome	11p13	194072	3
Prader-Willi syndrome	15q11.2	176270	4
Angelman syndrome	15q11.2	105830	
Rubinstein-Taybi syndrome	16p13.3	180849	2
Miller-Dieker syndrome	17p13.3	247200	4
Lissencephaly-1	17p13.3	607432	
Smith-Magenis syndrome	17p11.2	182290	4
Potocki-Lupski syndrome	17p11.2	610883	
Alagille syndrome	20p12.2	118450	2
DiGeorge syndrome	22q11.21	188400	7
22q11.2 microduplication syndrome	22q11.2	608363	
Phelan-McDermid syndrome	22q13	606232	2

Clinical background

Microdeletion and microduplication syndromes are defined as a group of clinically recognisable disorders characterised by a small (< 5 Mb) deletion or duplication of a chromosomal segment spanning one or multiple disease genes. The phenotype is the result of haploinsufficiency or overexpression of specific genes in the critical interval. Clinically well described syndromes, for which the involvement of multiple disease genes has been established or is strongly suspected, include DiGeorge syndrome (22q11 microdeletion), Williams-Beuren syndrome (7q11 microdeletion), Smith-Magenis Syndrome (17p microdeletion) and many more. Most patients with microdeletion/microduplication syndromes present with intellectual disability (ID), developmental delay (DD), congenital abnormalities and/or dysmorphic features.

Intellectual disability and developmental delay affects 1–3% of the population and results from extraordinary heterogeneous environmental, chromosomal and monogenic causes. Detailed analysis of the Online Mendelian Inheritance in Man (OMIM) database and literature searches revealed more than a thousand entries for ID and DD, and more than 290 genes involved in clinical phenotypes or syndromes, metabolic or neurological disorders characterised by ID/DD.

The genetic changes of microdeletions/duplications are often not detectable by the current band resolution using routine or high resolution karyotyping (2-5 Mb) but require the application of molecular cytogenetic techniques such as Fluorescence In Situ Hybridisation (FISH), MLPA or array Comparative Genomic Hybridisation (aCGH).

Exon numbering

The source of exon numbering used in the P064-C2 Coffalyser sheet is the exon numbering from the NM_ sequences as mentioned in the table below. 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.

Gene	NM_ sequence
ACTRT2	NM_080431.4
PEX10	NM_153818.1
TNFRSF4	NM_003327.4

Gene	NM_ sequence
GABRD	NM_000815.5
WHSC1	NM_001042424.3
TACC3	NM_006342.1

Gene	NM_ sequence
CTNND2	NM_001332.2
CLPTM1L	NM_030782.3
TERT	NM_198253.3

Gene	NM_ sequence	Gene	NM_ sequence	Gene	NM_ sequence
IRX4	NM_016358.2	*FSHB*	NM_000510.2	*CDC45*	NM_001178010.2
NSD1	NM_022455.5	*UBE3A*	NM_130838.1	*GNB1L*	NM_053004.2
TWIST1	NM_000474.4	*SNRPN*	NM_022807.5	*SNAP29*	NM_004782.4
ELN	NM_000501.4	*CREBBP*	NM_004380.3	*CLTCL1*	NM_007098.3
TRPS1	NM_014112.5	*TOM1L2*	NM_001033551.2	*DGCR8*	NM_022720.6
EIF3H	NM_003756.2	*RAI1*	NM_030665.4	*MED15*	NM_015889.3
EXT1	NM_000127.3	*METTL16*	NM_024086.3	*ZNF74*	NM_003426.2
PAX6	NM_000280.4	*PAFAH1B1*	NM_000430.4	*SHANK3*	NM_001372044.2
DCDC1	NM_181807.3	*JAG1*	NM_000214.3	*ARSA*	NM_000487.5

Probemix content

The SALSA MLPA Probemix P064-C2 Microdeletion Syndromes-1B contains 52 MLPA probes with amplification products between 130 and 483 nucleotides (nt). The probes detect sequences involved in a distinct subset of microdeletion and microduplication disorders (described above). 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 from peripheral blood, buccal swabs, (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or foetal blood, 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, intellectual disability and/or congenital anomalies. 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 table below shows the sample ID numbers from the Coriell Institute that have been tested with this P064-C2 probemix at MRC-Holland and can be used as positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID	Disorder	Affected probes
NA22991	1p36 deletion syndrome	Heterozygous deletion 404, 148, 315 and 136 nt probes
NA22601	Wolf-Hirschhorn syndrome	Heterozygous deletion 453, 199, 445 and 238 nt probes
NA14131	Cri-du-Chat syndrome	Heterozygous deletion 364, 268, 251, 372 and 413 nt probes
NA10160	Williams-Beuren syndrome	Heterozygous deletion 388, 339 and 469 nt probes
NA09888	Trichorhinophalangeal syndrome type 2	Heterozygous deletion 226, 130 and 274 nt probes
NA21887	Angelman syndrome	Heterozygous deletion 396, 219, 346 and 287 nt probes
NA06047	Miller-Dieker syndrome	Heterozygous deletion 436, 187, 323 and 292 nt probes
NA13476	Smith-Magenis syndrome	Heterozygous deletion 244, 421, 165 and 193 nt probes
NA17942	DiGeorge syndrome	Heterozygous deletion 205, 476, 331, 154, 211, 461 and 380 nt probes

Performance characteristics
Clinical performance is mainly dependent on the populations or cohort studied. According to literature, approximately 10-20% of patients with congenital anomalies, (neuro)developmental delay or intellectual disability tested with the P064 probemix show microdeletions or microduplications, which leads to a significant diagnostic yield in testing for intellectual disability syndromes and/or chromosomal imbalances.

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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 expected results for the probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication). In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication) may be obtained.

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.

P064-specific note

- There are no dedicated reference probes in this probemix but instead all probes 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. Data normalisation should be performed within one experiment.

Limitations of the procedure

- The P064 probemix has a limited number of probes for each specific chromosomal region and will therefore not detect all possible causes of the syndromes included. The detection rate may vary between syndromes, depending on the heterogeneity of the disorder.

- For Prader-Willi and Angelman syndromes, the P064 probemix can only be used to detect copy number changes of the 15q11.2 region. Probes for the detection of methylation changes at this locus are present in the ME028 Prader-Willi/Angelman probemix.
- 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. Furthermore, copy number changes detected by the P064 probemix can be confirmed by using a syndrome-specific MLPA probemix (see notes at Table 2).

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 SNVs 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 P064-C2 Microdeletion Syndromes-1B

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Syndrome detected
64-105	Control fragments – see table in probemix content section for more information		
130	**EIF3H probe** 13351-L14781	8q24.11	Trichorhinophalangeal syndrome type 2
136	**ACTRT2 probe** 14271-L22751	1p36.32	1p36 deletion syndrome
141	**CREBBP probe** 03088-L22752	16p13.3	Rubinstein-Taybi syndrome
148 «	**GABRD probe** 13354-L14784	1p36.33	1p36 deletion syndrome
154 «	**DGCR8 probe** 08475-L20946	22q11.21	DiGeorge / 22q11.2 duplication syndrome
159	**PAX6 probe** 06025-L20947	11p13	WAGR syndrome
165 «	**RAI1 probe** 16526-L20951	17p11.2	Smith-Magenis / Potocki-Lupski syndrome
170	**FSHB probe** 07075-L20952	11p14.1	WAGR syndrome
175	**DCDC1 probe** 07077-L20953	11p13	WAGR syndrome
181	**NSD1 probe** 01301-L20954	5q35.3	Sotos syndrome
187 «	**PAFAH1B1 probe** 15683-L20955	17p13.3	Miller-Dieker syndrome / Lissencephaly-1
193	**TOM1L2 probe** 04669-L20956	17p11.2	Smith-Magenis / Potocki-Lupski syndrome
199	**WHSC1 probe** 13764-L20957	4p16.3	Wolf-Hirschhorn syndrome
205	**CLTCL1 probe** 05462-L20958	22q11.21	DiGeorge / 22q11.2 duplication syndrome
211	**ZNF74 probe** 05927-L20959	22q11.21	DiGeorge / 22q11.2 duplication syndrome
219	**SNRPN probe** 11180-L30303	15q11.2	Prader-Willi / Angelman syndrome
226	**TRPS1 probe** 07371-L20961	8q23.3	Trichorhinophalangeal syndrome type 2
232	**NSD1 probe** 17561-L20962	5q35.3	Sotos syndrome
238	**WHSC1 probe** 06060-L17912	4p16.3	Wolf-Hirschhorn syndrome
244 «	**RAI1 probe** 16587-L20964	17p11.2	Smith-Magenis / Potocki-Lupski syndrome
251 «	**CLPTM1L probe** 13357-L23702	5p15.33	Cri-du-Chat syndrome
256 «	**TWIST1 probe** 04916-L20966	7p21.1	Saethre-Chotzen syndrome
264 «	**NSD1 probe** 14898-L20967	5q35.2	Sotos syndrome
268	**TERT probe** 08643-L23399	5p15.33	Cri-du-Chat syndrome
274	**EXT1 probe** 12567-L23738	8q24.11	Trichorhinophalangeal syndrome type 2
281	**JAG1 probe** 05989-L23739	20p12.2	Alagille syndrome
287	**UBE3A probe** 10879-L23740	15q11.2	Prader-Willi / Angelman syndrome
292 «	**PAFAH1B1 probe** 01925-L23741	17p13.3	Miller-Dieker syndrome / Lissencephaly-1
298	**CREBBP probe** 09883-L23742	16p13.3	Rubinstein-Taybi syndrome
306	**ARSA probe** 02707-L30304	22q13.33	Phelan-McDermid syndrome
315 ±	**PEX10 probe** 04678-L22755	1p36.32	1p36 deletion syndrome
323	**PAFAH1B1 probe** 07276-L22756	17p13.3	Miller-Dieker syndrome / Lissencephaly-1
331	**GNB1L probe** 07487-L22757	22q11.21	DiGeorge syndrome
339	**ELN probe** 01334-L14434	7q11.23	Williams-Beuren syndrome
346	**UBE3A probe** 10887-L20995	15q11.2	Prader-Willi / Angelman syndrome
355 «	**TWIST1 probe** 04915-L20996	7p21.1	Saethre-Chotzen syndrome
364	**TERT probe** 08653-L23271	5p15.33	Cri-du-Chat syndrome
372	**IRX4 probe** 15433-L17263	5p15.33	Cri-du-Chat syndrome
380	**SNAP29 probe** 16748-L23315	22q11.21	DiGeorge / 22q11.2 duplication syndrome
388 ±	**ELN probe** 13361-L20998	7q11.23	Williams-Beuren syndrome
396	**SNRPN probe** 12478-L20999	15q11.2	Prader-Willi / Angelman syndrome
404 «	**TNFRSF4 probe** 09198-L21007	1p36.33	1p36 deletion syndrome
413	**CTNND2 probe** 03073-L21008	5p15.2	Cri-du-Chat syndrome
421 ±	**RAI1 probe** 16591-L21009	17p11.2	Smith-Magenis / Potocki-Lupski syndrome
428 « +	**SHANK3 probe** 14193-L22758	22q13.33	Phelan-McDermid syndrome
436 ±	**METTL16 probe** 01924-L22759	17p13.3	Miller-Dieker syndrome / Lissencephaly-1
445	**WHSC1 probe** 06058-L05513	4p16.3	Wolf-Hirschhorn syndrome
453	**TACC3 probe** 15440-L21012	4p16.3	Wolf-Hirschhorn syndrome
461	**MED15 probe** 18308-L05816	22q11.21	DiGeorge / 22q11.2 duplication syndrome
469 ±	**ELN probe** 13365-L23317	7q11.23	Williams-Beuren syndrome
476	**CDC45 probe** 05463-L23743	22q11.21	DiGeorge / 22q11.2 duplication syndrome
483	**JAG1 probe** 03072-L21021	20p12.2	Alagille syndrome

« 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.
± SNP rs147723448 is known to influence the 388 nt probe signal. SNP rs537327331 is known to influence the 436 nt probe signal. SNPs rs372279226 and rs542051024 are known to influence the 469 nt probe signal. SNP rs763626682 could influence the 315 nt probe signal. SNP rs01842299 could influence the 421 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
+ The presence of salt in DNA samples can result in incomplete denaturation of CpG islands, which may result in false positive results: apparent deletions of this probe should be handled with care. Usually Coffalyser.Net issues a sample denaturation warning when the 88 nt and/or 96 nt D-fragments are too low. This SHANK3 probe targets an extremely GC-rich chromosomal area, and is affected by salt concentrations that not yet affect the control D- fragments, thus without the software issuing a warning. False positive results are more likely when DNA has been extracted by the Qiagen EZ1, M48 or M96 systems, as these leave a higher salt concentration in the sample. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration or other related technique).

SNVs located in the target sequence of a probe can influence probe hybridization 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. P064-C2 probes arranged according to chromosomal location

Table 2a. 1p36 deletion syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	02270-L01762	*TNFRSF18*		*P070 probe for 1p36*	*8 kb*
	02269-L01761	*TNFRSF4*		*P036 probe for 1p36*	*1 kb*
404 «	09198-L21007	*TNFRSF4*	01-001.14	AACTCCAGGCTT-GTAGCTGTCCAG	811 kb
148 «	13354-L14784	*GABRD*	01-001.95	TCATCGGAGGAC-ATCGTCTACTAC	382 kb
315 ±	04678-L22755	*PEX10*	01-002.33	AGTGGCTGGAGT-GGAGGAAGGAGG	597 kb
136	14271-L22751	*ACTRT2*	01-002.93	TGACGTGGAGAG-ACTCTGGAAGCA	

- SALSA MLPA probemix P147 contains more probes targeting 1p36 sequences.
- Deletions in the 1p36 region have been reported to be a frequent cause of developmental delay and intellectual disability with a frequency between 1:5,000 and 1:10,000 births. The majority of cases encompass terminal deletions that should also be detected by SALSA MLPA probemixes P036 and P070. Several interstitial deletions and complex rearrangements have been described.
- More information on 1p36 deletion syndrome can be found in OMIM 607872. Patients with 1p36 deletion syndrome present with typical craniofacial features, brachy/camptodactyly, short feet, and developmental delay and intellectual disability of variable degree. Hypotonia, seizures, structural brain abnormalities, and congenital heart defect may occur as well.

Table 2b. Wolf-Hirschhorn syndrome, 4p16.3

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	02005-L02047	*PIGG*		*P036 probe for 4p*	*1 kb*
	14440-L16146	*PIGG*		*P070 probe for 4p*	*1194 kb*
453	15440-L21012	*TACC3*	04-001.70	GGACAAAATGGA-TGACCCAAACTT	172 kb
199	13764-L20957	*WHSC1*	04-001.87	ACTGCGTTTTGA-GTCCCAGGAAAT	30 kb
445	06058-L05513	*WHSC1*	04-001.90	GCTGAGTGAGAA-GCAGAGAGCACG	48 kb
238	06060-L17912	*WHSC1*	04-001.95	CTTGGCATCATT-GTGACGTGTGTG	

- The most frequent cause is a terminal deletion of 4p16.3 that can also be detected by the telomeric probemixes P036 and P070.
- The WHS critical region is located approximately 1.9 Mb from the telomere and includes the *WHSC1* gene.
- More information on Wolf-Hirschhorn syndrome can be found in OMIM 194190. Phenotype includes a variable degree of developmental delay and intellectual disability, seizures and skeletal anomalies.

Table 2c. Cri-du-Chat syndrome, 5p15

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	02791-L02233	*CCDC127*		*P070 probe for 5p*	*109 kb*
	01723-L01327	*PDCD6*		*P036 probe for 5p*	*944 kb*

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
364	08653-L23271	*TERT*	05-001.31	GCACCAACATCT-ACAAGATCCTCC	35 kb
268	08643-L23399	*TERT*	05-001.35	GCTTCCTCAGGA-ACACCAAGAAGT	45 kb
251 «	13357-L23702	*CLPTM1L*	05-001.39	GAAAACCGTGCA-TTACCTGCCCAT	540 kb
372	15433-L17263	*IRX4*	05-001.93	GGACGGGGTCTT-CCACGACCCCAT	9854 kb
413	03073-L21008	*CTNND2*	05-011.79	CATCAGCCTCAG-AGAAGACGAGTT	

- The most frequent cause of the Cri-du-Chat syndrome is a terminal deletion of 5p15 that can also be detected by the telomeric probemixes P036 and P070. Interstitial deletions have also been described (Zhang et al. 2005).
- More information on Cri-du-Chat syndrome can be found in OMIM 123450. Clinical features include severe psychomotor retardation, intellectual disability and the characteristic cat-like cry.

Table 2d. Sotos syndrome, 5q35.3

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
264 «	14898-L20967	*NSD1*	05-176.49	ATATTGATGAGA-GCGATCGGCTCG	126 kb
232	17561-L20962	*NSD1*	05-176.62	TACCACGCCAAT-GACTTTTGCCTG	35 kb
181	01301-L20954	*NSD1*	05-176.65	AGCTTCACCTCA-TCAGGTCACACC	

- More probes for the *NSD1* gene are present in the P026 Sotos syndrome probemix.
- Of all *NSD1* mutations detected, ~10% (non-Japanese population) to ~45% (Japanese population) are complete gene deletions. Reciprocal duplications cause the opposite phenotype of Sotos syndrome (Franco et al. 2010).
- Distance from the *NSD1* gene to the 5q telomeric probes in P036 and P070 is approximately 3950 kb. Sotos syndrome is mainly caused by point mutations in the *NSD1* gene, which will not be detected by MLPA.
- More information on Sotos syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1479/ and in OMIM 117550. Sotos syndrome is characterised by excessive physical growth in infancy and macrocephaly, and may be accompanied by autism, mild intellectual disability and delayed motor development.

Table 2e. Saethre-Chotzen syndrome, 7p21.1

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
355 «	04915-L20996	*TWIST1*	07-019.12	AAGAGCCTCCAA-GTCTGCAGCTCT	2 kb
256 «	04916-L20966	*TWIST1* upstream	07-019.13	GCTGGAGAAATA-ACACTCGCCCTC	

- More probes for the *TWIST1* gene are present in the probemixes P054 FOXL2-TWIST1 and P080 Craniofacial.
- The majority of Saethre-Chotzen syndrome is caused by point mutations in the *TWIST1* gene, which cannot be detected by MLPA. Approximately 11-28% of Saethre-Chotzen patients have a deletion involving the *TWIST1* gene.
- More information on Saethre-Chotzen syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1189/ and in OMIM 101400. Phenotype includes coronal synostosis, facial dysmorphology and syndactyly. In contrast to patients with a *TWIST1* point mutation, patients with a (partial) deletion of *TWIST1* are often developmentally delayed.

Table 2f. Williams-Beuren syndrome / Williams-Beuren duplication syndrome, 7q11.23

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
388 ±	13361-L20998	*ELN*	07-073.09	GGCCTCGGAGCA-TTGAGAGACAGC	5 kb
339	01334-L14434	*ELN*	07-073.09	GGTGGAGTGGCT-GACGCTGCTGCA	20 kb
469 ±	13365-L23317	*ELN*	07-073.12	GAGACCCATCGT-TCAGAAATGGAA	

- More probes in the Williams-Beuren syndrome (WBS) region are present in the P029 WBS probemix. The majority (>90%) of the WBS patients have a 1.6 Mb deletion that includes the *ELN* and *LIMK1* genes. A deletion of the 7q11.23 chromosomal region, including the *ELN* gene is found in approximately 90-95% of the clinically typical WBS patients but in a lower percentage of atypical cases.
- Besides deletions of the WBS region, some duplications have also been described, giving rise to the Williams-Beuren duplication syndrome (OMIM 609757).
- More information on Williams-Beuren syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1249/ and in OMIM 194050. Williams-Beuren syndrome is characterised by supravalvular aortic stenosis (SVAS), infantile hypercalcemia, intellectual disability, and distinctive facial features.

Table 2g. Trichorhinophalangeal syndrome type 2, 8q24.11-q24.13

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
226	07371-L20961	*TRPS1*	08-116.70	CCGTTCTGTGTT-TTCTGGTGTGCT	1136 kb

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
130	13351-L14781	*EIF3H*	08-117.84	TAGATGGCCTTG-TGAGTGCTGTTC	1081 kb
274	12567-L23738	*EXT1*	08-118.92	GGTGATAATGTT-AAACCCACTTAA	

- More probes for Trichorhinophalangeal syndrome type 2 are present in the P215 EXT probemix.
- Most Trichorhinophalangeal syndrome type 2 (TRPS2) patients have a microdeletion that includes the *TRPS1* and *EXT1* genes. TRPS2 is also known as Langer-Giedion Syndrome (LGS).
- More information on TRPS2 can be found in OMIM 150230. Phenotype includes multiple dysmorphic facial features, multiple cartilaginous exostoses, redundant skin, sparse scalp hair and mild to moderate intellectual disability.

Table 2h. WAGR syndrome, 11p13

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
170	07075-L20952	*FSHB*	11-030.21	GAACTAACTGCA-GCAGTCTTCTGG	1074 kb
175	07077-L20953	*DCDC1*	11-031.29	CAGCAGTATCAG-AAGGGTCAGGAC	503 kb
159	06025-L20947	*PAX6*	11-031.79	AAACTCTCACCA-GCAACTCCTTTA	

- More probes for *PAX6* are present in the P219 PAX6 probemix.
- More information on WAGR syndrome can be found in OMIM 194072. Children affected with WAGR syndrome are predisposed to develop **W**ilms tumours, **A**niridia, **G**enitourinary anomalies (or **G**onadoblastoma), and mental **R**etardation.

Table 2i. Prader-Willi / Angelman syndrome, 15q11.2

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	07291-L08858	*MKRN3*	*P036 probe for “15q”*		*120 kb*
	04026-L01542	*NDN*	*P070 probe for “15q”*		*1234 kb*
396	12478-L20999	*SNRPN*	15-022.72	AACCTGGGTTAG-AGAAAATTACAG	34 kb
219	11180-L30303	*SNRPN*	15-022.75	AAGTCTGGCGTA-TTTGCATTGATT	385 kb
346 #	10887-L20995	*UBE3A*	15-023.14	CGGAATACTCAA-GCAAAGAAAAAC	66 kb
287	10879-L23740	*UBE3A*	15-023.20	ATGGGAGATAGG-AACATACCTACT	

- More probes for the Prader-Willi / Angelman region, including probes for the detection of methylation changes, are present in the ME028 PWS/AS probemix.
- The majority of the Prader-Willi and Angelman patients have a copy number change of the 15q11.2 region that should be detected by this P064 probemix. However, a considerable number of patients (~30%) have a change in methylation status of the 15q11.2 region that can be detected by the ME028 PWS/AS probemix, but not with this P064 probemix.
- More information on Prader-Willi syndrome (PWS) can be found on http://www.ncbi.nlm.nih.gov/books/NBK1330/ and in OMIM 176270. More information on Angelman syndrome (AS) can be found on http://www.ncbi.nlm.nih.gov/books/NBK1144/ and in OMIM 105830. PWS and AS are clinically distinct complex disorders. They both have characteristic neurologic, developmental, and behavioural phenotypes plus other structural and functional abnormalities. However, the cognitive and neurologic impairment is more severe in AS, including seizures and ataxia. The behavioural and endocrine disorders are more severe in PWS, including obsessive-compulsive symptoms and hypothalamic insufficiency.

Table 2j. Rubinstein-Taybi syndrome, 16p13.3

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
141	03088-L22752	*CREBBP*	16-003.72	GCTTGTAGCATT-GTGAGAGCATCA	83 kb
298	09883-L23742	*CREBBP*	16-003.80	CCAACGTGCCAA-ATATGGTAAGTT	

- More probes for the *CREBBP* gene are present in the P313 CREBBP probemix.
- The 16p13.3 deletion syndrome (OMIM 610543) is caused by larger deletions that include the *CREBBP* gene and leads to a severe form of Rubinstein-Taybi syndrome.
- Only a minority of Rubinstein-Taybi patients (~10%) can be detected with the use of these two probes, since most patients have a point mutation in the *CREBBP* or *EP300* gene.
- More information on Rubinstein-Taybi syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1526/ and in OMIM 180849. Phenotype includes distinctive facial features, broad and often angulated thumbs and great toes, short stature, and moderate to severe intellectual disability.

Table 2k. Miller-Dieker syndrome / Lissencephaly-1, 17p13.3

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
436 ±	01924-L22759	*METTL16*	17-002.36	CGGCTGCTTTAA-GATTCTAGGGTT	82 kb

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
187 «	15683-L20955	*PAFAH1B1*	17-002.44	ACACGGGAGTCT-AGGGAGCGAGAA	44 kb
323 #	07276-L22756	*PAFAH1B1*	17-002.49	TACCACTATATC-AGATAAGCTTGA	43 kb
292 # «	01925-L23741	*PAFAH1B1*	17-002.53	ACTGGCAGCGTA-GATCAAACAGTA	

- More probes for *PAFAH1B1* and other genes in the Miller-Dieker region are present in the P061 Lissencephaly probemix.
- The majority of Lissencephaly-1 patients and nearly all Miller-Dieker patients have a chromosomal deletion that includes the *PAFAH1B1* gene. Several patients with a duplication in the Miller-Dieker region have been described, presenting with a large variety of clinical features (OMIM 613215).
- More information on these syndromes can be found on http://www.ncbi.nlm.nih.gov/books/NBK5189/, in OMIM 607432 (Lissencephaly-1), and OMIM 247200 (Miller-Dieker syndrome). Phenotype includes cortical malformations, typical facial features, and severe neurologic abnormalities.

Table 2l. Smith-Magenis syndrome / Potocki-Lupski syndrome, 17p11.2

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
244 «	16587-L20964	*RAI1*	17-017.53	GTGATGAGCCGA-GGCGGGTTCGGA	111 kb
421 ±	16591-L21009	*RAI1*	17-017.64	TCTTTTCGAGAA-AGGTGTGGTTTC	18 kb
165 «	16526-L20951	*RAI1*	17-017.65	CAGCGCTAGATT-TCGTGTACAAAA	162 kb
193	04669-L20956	*TOM1L2*	17-017.82	GACAGAGGTGTA-ACGACCAATAGG	

- More probes for the Smith-Magenis region are present in the P369 Smith-Magenis probemix.
- The majority (90%) of Smith-Magenis syndrome (SMS) is caused by a 3.7 Mb interstitial deletion on chromosome 17p11.2. A duplication of the same region leads to a milder phenotype, known as Potocki-Lupski syndrome (PTLS).
- More information on SMS and PTLS can be found on http://www.ncbi.nlm.nih.gov/books/NBK1310/, in OMIM 182290, and in OMIM 610883. SMS is characterised by distinctive physical features, developmental delay, cognitive impairment, behavioural abnormalities, and mild to moderate intellectual disability. PTLS is characterised by hypotonia, failure to thrive, intellectual disability, pervasive developmental disorders, and congenital anomalies.

Table 2m. Alagille syndrome, 20p12.2

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
281	05989-L23739	*JAG1*	20-010.57	GGAGCGACCTGT-GTGGATGAGATC	31 kb
483	03072-L21021	*JAG1*	20-010.60	TTTTCCAGTCGT-GCATGCTCCAAT	

- Probes for confirmation are available in the P184 JAG1 probemix.
- The majority (~89%) of Alagille syndrome (ALGS) is caused by a point mutation in the *JAG1* gene. Approximately 7% of the affected individuals have a deletion of chromosome 20p12.2 involving this gene.
- More information on Alagille syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1273/ and in OMIM 118450. The clinical features are highly variable and may include cholestasis, congenital cardiac defects, skeletal abnormalities, eye abnormalities, and typical facial features.

Table 2n. DiGeorge / 22q11.2 duplication syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	02725-L16344	*IL17RA*	*P070 probe for 22q11*		*647 kb*
	01740-L01310	*BID*	*P036 probe for 22q11*		*1015 kb*
205	05462-L20958	*CLTCL1*, region AB	22-017.62	TGTTGCCTTGGT-GACCGAGACCGC	226 kb
476	05463-L23743	*CDC45*, region AB	22-017.85	ATGTTCGTGTCC-GATTTCCGCAAA	309 kb
331	07487-L22757	*GNB1L*, region AB	22-018.16	CGGGATCGCCGA-GGTCACGATCCG	297 kb
154 «	08475-L20946	*DGCR8*, region AB	22-018.45	GGTAATGGACGT-TGGCTCTGGTGG	626 kb
211	05927-L20959	*ZNF74*, region BC	22-019.08	CAGGCAGATTAT-TCCTCGATGCTG	187 kb
461	18308-L05816	*MED15*, region BC	22-019.27	TGGCATTTGGAT-GAAGACACAGGT	299 kb
380	16748-L23315	*SNAP29*, region CD	22-019.57	GTATCCACTTAC-CTGTATCATCCA	

- More probes for the 22q11 DiGeorge region are present in the P250 DiGeorge probemix.
- Deletions in 22q11 are the most frequent cause of DiGeorge syndrome. These 22q11 deletions can be variable in size. The majority (~85%) include the AB, BC and CD regions, although some deletions are smaller (AB only) or larger.
- Cat eye syndrome patients can be detected with the probes in the P036 and P070 telomere probemixes, but not by the probes in this P064 mix.
- More information on DiGeorge syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1523/ and in OMIM 188400. A wide variety of clinical symptoms are known to be associated with DiGeorge syndrome, including congenital heart disease, palatal abnormalities, characteristic facial features, learning difficulties, immune deficiency, and hypocalcaemia.

Table 2o. Phelan-McDermid syndrome, 22q13

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
306	02707-L30304	*ARSA*	22-049.41	GGAGGATCAGAT-CTCCGCTCGAGA	78 kb
428 « +	14193-L22758	*SHANK3*	22-049.49	ACAGCTGAGCTC-GAGGAACTTGGT	62 kb
	01762-L08761	*RABL2B*	*P036 probe for 22q13*		

- More probes in the Phelan-McDermid region are present in the P188 22q13 probemix.
- The *SHANK3* gene is suspected to be responsible for at least part of the Phelan-McDermid syndrome phenotype. The *RABL2B* probe in P036 is located between *SHANK3* and the 22q telomere. The *ARSA* probes in P064 and P070 detect the same sequence.
- More information on Phelan-McDermid syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1198/ and in OMIM 606232. Phenotype includes neonatal hypotonia, global developmental delay, normal to accelerated growth, absent to severely delayed speech, autistic behaviour, and minor dysmorphic features. Most individuals have moderate to profound intellectual disability.

[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.
± SNP rs147723448 is known to influence the 388 nt probe signal. SNP rs537327331 is known to influence the 436 nt probe signal. SNPs rs372279226 and rs542051024 are known to influence the 469 nt probe signal. SNP rs763626682 could influence the 315 nt probe signal. SNP rs01842299 could influence the 421 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
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.
+ The presence of salt in DNA samples can result in incomplete denaturation of CpG islands, which may result in false positive results: apparent deletions of this probe should be handled with care. Usually Coffalyser.Net issues a sample denaturation warning when the 88 nt and/or 96 nt D-fragments are too low. This SHANK3 probe targets an extremely GC-rich chromosomal area, and is affected by salt concentrations that not yet affect the control D- fragments, thus without the software issuing a warning. False positive results are more likely when DNA has been extracted by the Qiagen EZ1, M48 or M96 systems, as these leave a higher salt concentration in the sample. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration or other related technique).

SNVs located in the target sequence of a probe can influence probe hybridization 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.

Notes

- More information on human genes and genetic disorders can be found on the OMIM website: https://www.ncbi.nlm.nih.gov/omim.
- Explanation for the column named 'MV36/hg18': 01-001.14 indicates that a probe is on chromosome 1, at 1.14 Mb distance from the p-telomere, according to NCBI Build 36/hg18 reference sequence.

Related SALSA MLPA probemixes

P036/P070 Subtelomeres	These probemixes contain probes for subtelomeric regions.
P106 X-linked ID	Contains probes for various genes involved in X-linked intellectual disability.
P245 Microdeletion Syndromes-1A	Contains probes for 1p36 deletion, 2p16.1-p15 microdeletion, 2q23.1 microdeletion/microduplication, Glass, 3q29 microdeletion, 3q29 microduplication, Wolf-Hirschhorn, Cri-du-Chat, Sotos, Williams-Beuren, Williams-Beuren duplication, Trichorhinophalangeal syndrome type 2, 9q22.3 microdeletion, DiGeorge type 2, Prader-Willi, Angelman, Witteveen-Kolk/15q24 microdeletion, Rubinstein-Taybi, Miller-Dieker, Lissencephaly type 1, Smith-Magenis, Potocki-Lupski, NF1

	microdeletion, Koolen-de Vries, 17q21.31 microduplication, DiGeorge, 22q11.2 microduplication, Distal 22q11.2 deletion, Phelan-McDermid, Rett, and MECP2 duplication syndrome.
P297 Microdeletion Syndromes-2	Contains probes for microdeletion syndromes on 1q21.1, 3q29, 15q13, 15q24, 16p13-11, and 17q12.

Several MLPA probemixes for specific syndromes are available, such as P250 DiGeorge, ME028 Prader-Willi/Angelman, P061 Lissencephaly and P029 WBS. Please see Table 2 or on www.mrcholland.com.

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P064 product history	
Version	*Modification*
C2	Small change in sequence of two probes.
C1	Probes for several extra microdeletion syndromes have been included, and a large number of the probes have been replaced.
B3	The 88 and 96 nt control fragments have been replaced and two control fragments at 100 (X chromosome) and 105 nt (Y chromosome) have been included (QDX2).
B2	*UBE3A* probe at 400 nt has been replaced by a new *UBE3A* probe. In addition, the DNA Denaturation control fragments (D-fragments) at 88 and 96 nt have been added.
B1	Nine probes have been replaced.
A	First release.

Implemented changes in the product description
Version C2-07 – 30 August 2023 (04P) - Langer-Giedion Syndrome is now called Trichorhinophalangeal syndrome type 2 throughout document, because this is the new standard in the OMIM database. - Warning for hypersensitivity to salt of the SHANK3 probe has been added to Table 1 and 2. - Section *Related SALSA MLPA Probemixes* on P245 adjusted. - Various minor textual and layout changes.
Version C2-06 – 23 February 2023 (04P) - Minor correction to the intended purpose was made to align with the Intended Purpose in the Technical File made June 2021: *…developmental delay and/or intellectual disability syndromes* was changed to *…developmental delay, intellectual disability and/or congenital anomalies.* - Updated NM_sequences of multiple genes to align with Coffalyser exon numbering. - Minor updates were made to Table 1 and 2: note for what changed in version C2 was removed from Table 1; background information in notes below Tables 2a-o was updated. - Section *Related SALSA MLPA probemixes* updated for P297. - Curated the section *Selected publications*. - Various minor textual and layout changes. - Implemented changes for version C2-05 were corrected (second point below).
Version C2-05 – 21 July 2021 (04P) - Product description restructured and adapted to a new template. - Recommendation to analyse male and female samples separately was removed as it does not apply to this product. - UK has been added to the list of countries in Europe that accept the CE mark. - Section 'exon numbering' added. - Removed information on P228, P371, P372, P373 and P374, as these products have been discontinued. - Warning removed on the influence of the SNP rs202220730 for probe 01301-L20954, as internal testing showed no effect on probe ratio. - Warnings added to Table 1 and 2 on the influence of the SNPs rs147723448, rs537327331, rs372279226, rs542051024, rs01842299 and rs763626682.
Version C2-04 – 8 November 2019 (02P) - Product description restructured and adapted to a new template. - Product is now registered for IVD use in Israel. - Information on data analysis and normalisation is added to indicate that there are no dedicated reference probes but instead all peaks are used for normalisation. - Warning added to Table 1 on the influence of the SNP rs202220730 for probe 01301-L20954.
Version C2-03 – 31 January 2019 (04) - Product is now registered for IVD use in Morocco.

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*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.