Product Description
SALSA® MLPA® Probemix P245-B1 Microdeletion Syndromes-1A

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 17.

Catalogue numbers
- **P245-025R:** SALSA® MLPA® Probemix P245 Microdeletion Syndromes-1A, 25 reactions
- **P245-050R:** SALSA® MLPA® Probemix P245 Microdeletion Syndromes-1A, 50 reactions
- **P245-100R:** SALSA® MLPA® Probemix P245 Microdeletion Syndromes-1A, 100 reactions

SALSA® MLPA® Probemix P245 Microdeletion Syndromes-1A (hereafter: P245 Microdeletion Syndromes-1A) is to be used in combination with:

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

Volumes and ingredients

Volumes			Ingredients
P245-025R	**P245-050R**	**P245-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

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

Storage and handling

Recommended storage conditions		

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

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

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

Data normalisation should be performed within one experiment. **It is required to analyse male and female samples separately**, *i.e.* only use male reference samples for the analysis of male test samples and only use female reference samples for the analysis of female test samples.

Please note that many regions targeted by P245 Microdeletion Syndromes-1A, and in particular the *MECP2*, *TRPS1* and *CREBBP* genes, are extremely GC-rich and are therefore difficult to denature. The use of DNA samples containing 20 mM or more salt can result in false positive deletion results. In particular, the use of Qiagen EZ1, M48 and M96 systems frequently results in DNA denaturation problems as these systems can result in very high salt concentrations. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration). When using silica column based DNA purification, salt concentrations can often be reduced by inclusion of a wash step with 85% ethanol before the elution step.

A low signal of the 88 nt and 96 nt DNA denaturation control fragments provides a warning for incomplete DNA denaturation. Please note that at certain salt concentrations, MECP2, TRPS1 and CREBBP probes may show reduced probe signals even in the absence of a denaturation warning in the Coffalyser.Net software.

Intended purpose

The SALSA MLPA Probemix P245 Microdeletion Syndromes-1A 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. P245 Microdeletion Syndromes-1A 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 P245 Microdeletion Syndromes-1A 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 P245 Microdeletion Syndromes-1A			
Syndrome	*Genetic locus*	*OMIM*	*Number of probes*
1p36 deletion syndrome	1p36	607872	3
2p16.1-p15 microdeletion syndrome	2p16.1-p15	612513	2
2q23.1 microdeletion/microduplication syndrome	2q23.1	156200	2
Glass syndrome	2q32-q33	612313	2
3q29 microdeletion syndrome	3q29	609425	2
3q29 microduplication syndrome	3q29	611936	
Wolf-Hirschhorn syndrome	4p16.3	194190	2
Cri-du-Chat syndrome	5p15	123450	2
Sotos syndrome	5q35.3	117550	2
Williams-Beuren syndrome	7q11.23	194050	2
Williams-Beuren duplication syndrome	7q11.23	609757	
Langer-Giedion syndrome	8q24.11-q24.13	150230	2

9q22.3 microdeletion syndrome	9q22.3	-	2
DiGeorge syndrome/velocardiofacial syndrome complex 2	10p14-p13	601362	1
Prader-Willi syndrome	15q11.2	176270	3
Angelman syndrome	15q11.2	105830	
Witteveen-Kolk* / 15q24 microdeletion syndrome	15q24	613406	2
Rubinstein-Taybi syndrome	16p13.3	180849	1
Miller-Dieker syndrome	17p13.3	247200	2
Lissencephaly-1	17p13.3	607432	
Smith-Magenis syndrome	17p11.2	182290	3
Potocki-Lupski syndrome	17p11.2	610883	
NF1 microdeletion syndrome	17q11.2	613675	2
Koolen-de Vries syndrome	17q21.31	610443	2
17q21.31 microduplication syndrome	17q21.31	613533	
22q11.2 deletion syndrome	22q11.21	188400	5
22q11.2 microduplication syndrome	22q11.2	608363	
Distal 22q11.2 deletion syndrome	22q11.2	611867	
Phelan-McDermid syndrome	22q13	606232	2
Rett syndrome	Xq28	312750	3
MECP2 duplication syndrome	Xq28	300260	

* Please note that the *SIN3A* gene, which has been described as the critical gene in Witteveen-Kolk syndrome, is not covered by the probes in P245 Microdeletion Syndromes-1A.

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 22q11.2 deletion syndrome (22q11 microdeletion), Williams-Beuren syndrome (7q11 microdeletion), Neurofibromatosis type 1 (17q11 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.

ID and DD 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 exon numbering used in this P245-B1 Microdeletion Syndromes-1A product description 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
TNFRSF4	NM_003327.4
GNB1	NM_002074.5
GABRD	NM_000815.5
PEX13	NM_002618.4
REL	NM_002908.4
MBD5	NM_018328.5
SATB2	NM_015265.4
DLG1	NM_004087.2
WHSC1	NM_001042424.3
LETM1	NM_012318.3
SEMA5A	NM_003966.3
TERT	NM_198253.3
NSD1	NM_022455.5
ELN	NM_000501.4

Gene	NM_ sequence
TRPS1	NM_014112.5
EXT1	NM_000127.3
FANCC	NM_000136.3
PTCH1	NM_000264.5
GATA3	NM_001002295.2
SNRPN	NM_022807.5
UBE3A	NM_000462.5
SEMA7A	NM_003612.5
CYP1A1	NM_001319217.2
CREBBP	NM_004380.3
RAI1	NM_030665.4
LLGL1	NM_004140.4
DRC3	NM_031294.4
PAFAH1B1	NM_000430.4

Gene	NM_ sequence
NF1	NM_000267.3
MAPT	NM_016835.4
KANSL1	NM_001193466.2
GP1BB	NM_000407.5
PPIL2	NM_014337.4
SNAP29	NM_004782.4
CLDN5	NM_001130861.1
RTDR1	NM_014433.3
RABL2B	NM_007081.4
SHANK3	NM_001372044.2
DMD	NM_004006.3
MECP2	NM_004992.4

Probemix content

P245-B1 Microdeletion Syndromes-1A contains 50 MLPA probes with amplification products between 130 and 499 nucleotides (nt). The probes detect sequences involved in a distinct subset of microdeletion and microduplication disorders (described above). Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains ten quality control fragments generating amplification products between 64 and 118 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and two chromosome Y-specific fragments (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)
105-118	Y-fragments (Y chromosome specific)

MLPA technique

The principles of MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation

Internal validation using 16 different DNA samples from healthy individuals of the same sex is required, in particular when using MLPA for the first time, or when changing the sample type or the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens

Extracted DNA 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. MRC Holland has tested and can recommend the following extraction methods:

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

All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of DD, ID and/or congenital anomalies. It is required to use samples of the same sex to facilitate interpretation. 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 P245-B1 Microdeletion Syndromes-1A at MRC-Holland and can be used as positive control samples. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

Sample ID	Disorder	Affected probes
NA22995	1p36 deletion syndrome	Deletion 130, 178 and 160 nt probes
NA11213	Glass syndrome	Deletion 485 and 391 nt probes
NA11428	3q29 microduplication syndrome	Duplication 355 and 422 nt probes
NA04126	Wolf-Hirschhorn syndrome	Deletion 232 and 454 nt probes
NA16593	Cri-du-Chat syndrome	Deletion 283 nt probe
NA13464	Williams-Beuren syndrome	Deletion 364 and 315 nt probes
NA20375	Angelman syndrome	Deletion 244, 300 and 166 nt probes
NA09208	Miller-Dieker syndrome	Deletion 142 and 238 nt probes
NA13476	Smith-Magenis syndrome	Deletion 471, 278 and 307 nt probes
NA02944	22q11.2 deletion syndrome	Deletion 196 and 208 nt probes
NA23635	Rett syndrome	Deletion 184 nt probe
NA23675	MECP2 duplication syndrome	Duplication 148, 184 and 202 nt probes
NA23676	MECP2 duplication syndrome	Duplication 148, 184 and 202 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)DD or ID tested with P245 Microdeletion Syndromes-1A show microdeletions or microduplications, which leads to a significant diagnostic yield in testing for ID syndromes and/or chromosomal imbalances.

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

Interpretation of results

The expected results for the probes detecting autosomal sequences 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 same results can be expected for the X-chromosome-specific probes in female samples. For the X-chromosome-specific probes in male samples, expected allele copy numbers are 1 (normal), 0 (deletion) or 2 (duplication).

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results when **reference samples of the same sex** have been used:

Copy number status		Final ratio (FR)
Autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication/gain		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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 Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: https://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript

variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- 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.

P245 Microdeletion Syndromes-1A specific notes:

- 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. It is recommended to analyse male and female samples separately and only use male reference samples for the analysis of male test samples and only use female reference samples for the analysis of female test samples.

Limitations of the procedure
- P245 Microdeletion Syndromes-1A 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, P245 Microdeletion Syndromes-1A 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 SALSA MLPA Probemix ME028 Prader-Willi/Angelman.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results
Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism in sequence data indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

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 https://varnomen.hgvs.org.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of two probes that are not consecutive in location) to MRC Holland: info@mrcholland.com.

Table 1. P245-B1 Microdeletion Syndromes-1A

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Syndrome detected
64-118	Control fragments – see table in probemix content section for more information		
130 «	**TNFRSF4** probe 02269-L01761	1p36.33	1p36 deletion syndrome
136	**GATA3** probe 07632-L07317	10p14	DiGeorge syndrome/velocardiofacial syndrome complex 2
142	**PAFAH1B1** probe 04120-L03532	17p13.3	Miller-Dieker syndrome / Lissencephaly-1
148 «	**MECP2** probe 09310-L13824	**Xq28**	Rett syndrome / *MECP2* duplication syndrome
154	**NSD1** probe 02595-L23366	5q35.3	Sotos syndrome
160 «	**GABRD** probe 04690-L04068	1p36.33	1p36 deletion syndrome
166	**UBE3A** probe 10877-L11547	15q11.2	Prader-Willi / Angelman syndrome
172 «	**CREBBP** probe 03087-L02487	16p13.3	Rubinstein-Taybi syndrome
178	**GNB1** probe 02890-L02511	1p36.33	1p36 deletion syndrome
184 «	**MECP2** probe 15319-L17592	**Xq28**	Rett syndrome / *MECP2* duplication syndrome
190	**SEMA7A** probe 18316-L23369	15q24.1	Witteveen-Kolk / 15q24 microdeletion syndrome
196	**CLDN5** probe 01218-L06270	22q11.21	22q11.2 deletion syndrome / 22q11.2 duplication syndrome
202 «	**MECP2** probe 03409-L16570	**Xq28**	Rett syndrome / *MECP2* duplication syndrome
208 « ±	**GP1BB** probe 05464-L15184	22q11.21	22q11.2 deletion syndrome / 22q11.2 duplication syndrome
214	**MBD5** probe 15311-L17110	2q23.1	2q23.1 microdeletion / microduplication syndrome
220	**PPIL2** probe 07530-L22697	22q11.21	Distal 22q11.2 deletion syndrome
226 ±	**REL** probe 17474-L22693	2p16.1	2p16.1-p15 microdeletion syndrome
232 «	**LETM1** probe 04190-L05920	4p16.3	Wolf-Hirschhorn syndrome
238	**PAFAH1B1** probe 16348-L22830	17p13.3	Miller-Dieker syndrome / Lissencephaly-1
244	**SNRPN** probe 12178-L13826	15q11.2	Prader-Willi / Angelman syndrome
252 «	**SHANK3** probe 12031-L13828	22q13.33	Phelan-McDermid syndrome
260	**NF1** probe 11732-L13830	17q11.2	*NF1* microdeletion syndrome
265 «	**RTDR1** probe 08484-L22698	22q11.22	Distal 22q11.2 deletion syndrome
272	**MAPT** probe 08365-L22699	17q21.31	Koolen-de Vries syndrome
278	**DRC3** probe 01452-L20745	17p11.2	Smith-Magenis / Potocki-Lupski syndrome
283	**SEMA5A** probe 14265-L22700	5p15.2	Cri-du-Chat syndrome
292	**DMD** probe 01411-L23371	**Xp21.1**	X-chromosome copy number
300	**SNRPN** probe 01318-L23196	15q11.2	Prader-Willi / Angelman syndrome
307 «	**LLGL1** probe 01453-L22689	17p11.2	Smith-Magenis / Potocki-Lupski syndrome
315 «	**ELN** probe 16349-L22813	7q11.23	Williams-Beuren syndrome
323	**PTCH1** probe 03702-L22814	9q22.32	9q22.3 microdeletion syndrome
331 ∫	**CYP1A1** probe 06811-L22815	15q24.1	Witteveen-Kolk / 15q24 microdeletion syndrome
339	**NF1** probe 02507-L22694	17q11.2	*NF1* microdeletion syndrome
346	**KANSL1** probe 18172-L22729	17q21.31	Koolen-de Vries syndrome
355	**DLG1** probe 08395-L08249	3q29	3q29 microdeletion/microduplication syndrome
364	**ELN** probe 01336-L00878	7q11.23	Williams-Beuren syndrome
373	**SNAP29** probe 16748-L19368	22q11.21	22q11.2 deletion syndrome / 22q11.2 duplication syndrome
382	**RABL2B** probe 06734-L05558	22q13.33	Phelan-McDermid syndrome
391	**SATB2** probe 15315-L17114	2q33.1	Glass syndrome
401 «	**TRPS1** probe 03081-L07411	8q23.3	Langer-Giedion syndrome
411	**MBD5** probe 15313-L22691	2q23.1	2q23.1 microdeletion/microduplication syndrome

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Syndrome detected
422	**DLG1** probe 08401-L15187	3q29	3q29 microdeletion/microduplication syndrome
429	**EXT1** probe 15322-L17698	8q24.11	Langer-Giedion syndrome
436	**FANCC** probe 04460-L22816	9q22.32	9q22.3 microdeletion syndrome
445	**TERT** probe 03761-L22817	5p15.33	Cri-du-Chat syndrome
454	**WHSC1** probe 10633-L14379	4p16.3	Wolf-Hirschhorn syndrome
462	**NSD1** probe 02600-L15191	5q35.3	Sotos syndrome
471	**RAI1** probe 11730-L15192	17p11.2	Smith-Magenis / Potocki-Lupski syndrome
485	**SATB2** probe 15318-L19750	2q33.1	Glass syndrome
499	**PEX13** probe 09870-L15194	2p15	2p16.1-p15 microdeletion syndrome

« Probe located within, 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.
± SNV rs749912012 could influence the 208 nt probe signal. SNV rs747391156 could influence the 226 nt probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
∫ Probe is very sensitive to pipetting mistakes.

Table 2. P245-B1 probes arranged according to chromosomal location

Table 2a. 1p36 deletion syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	02270-L01762	*TNFRSF18*	*P070 probe for 1p36*		*8 kb*
130 «	02269-L01761	TNFRSF4	01-001.14	GCCGGCCAGCAA-TAGCTCGGACGC	609 kb
178	02890-L02511	GNB1	01-001.75	CTAAGATCGGAA-GATGAGTGAGCT	200 kb
160 «	04690-L04068	GABRD	01-001.95	CGGCGACTACGT-GGGCTCCAACCT	

- SALSA MLPA Probemix P147 1p36 contains more probes targeting 1p36 sequences.
- Deletions in the 1p36 region have been reported to be a frequent cause of DD and ID 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 Probemix P036 Subtelomeres Mix 1 and SALSA MLPA Probemix P070 Subtelomeres Mix 2B. Several interstitial deletions and complex rearrangements have been described. The *TNFRSF4* probes in P245 Microdeletion Syndromes-1A and P036 Subtelomeres Mix 1 detect the same sequence.
- 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 DD and ID of variable degree. Hypotonia, seizures, structural brain abnormalities, and congenital heart defect may occur as well.

Table 2b. 2p16.1-p15 microdeletion syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
226 ±	17474-L22693	REL	02-061.00	TATCACAGAACC-CGTAACAGTAAA	127 kb
499	09870-L15194	PEX13	02-061.13	TGAGGATGACCA-TGTAGTTGCCAG	

- The interstitial 2p16.1-p15 microdeletion syndrome has been described first by Rajcan-Separovic et al. (2007). Until now, several patients with variable deletion lengths on chromosome 2p16.1-p15 have been described.
- More information on 2p16.1-p15 microdeletion syndrome can be found in OMIM 612513. Phenotype includes delayed psychomotor development, ID, and dysmorphic features. Many patients have behavioural disorders, as well as structural brain abnormalities.

Table 2c. 2q23.1 microdeletion/microduplication syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
214	15311-L17110	MBD5	02-148.93	CCAGCTATACAA-GTTCCTGTGGGT	54 kb
411	15313-L22691	MBD5	02-148.99	CTGGAGATCTTC-CTCCTCTTGGGT	

- Information on the interstitial 2q23.1 microdeletion syndrome is e.g. present in Mullegama et al. (2015). Microduplications of 2q23.1 have e.g. been described by Chung et al. (2012). Disruption of the *MBD5* gene is considered to be causative for the phenotype.
- More information on 2q23.1 microdeletion/microduplication syndrome can be found in OMIM 156200. Information on *MBD5* haploinsufficiency can be found in https://www.ncbi.nlm.nih.gov/books/NBK390803/. Patients with 2q23.1 microdeletion or microduplication syndrome often present with ID, speech impairment, epilepsy, short stature, characteristic facial features, and autism.

Table 2d. Glass syndrome (2q32-q33 microdeletion syndrome)

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
485	15318-L19750	SATB2	02-199.90	TGCCATTTATGA-CGAGATCCAACA	110 kb
391	15315-L17114	SATB2	02-200.01	AGAGAAGAACAC-GCCGAGTTTGTC	

- Information on the interstitial 2q32-q33 microdeletion syndrome is e.g. present in Balasubramanian et al. (2011). Glass syndrome patients with variable deletion lengths have been described. Almost all of them involve the *SATB2* gene, which is considered the main cause of the phenotype.
- More information on Glass syndrome can be found in OMIM 612313. Phenotype includes severe ID, seizures, growth retardation, and tooth abnormalities.

Table 2e. 3q29 microdeletion/microduplication syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
355	08395-L08249	DLG1	03-198.28	CTATGAAAGACA-GGATAAATGATG	231 kb
422	08401-L15187	DLG1	03-198.51	CAGCTCAGAAGT-TCCATAGAACGG	250 kb
	02013-L02052	*BDH1*	*P036 probe for 3q*		*126 kb*
	02690-L02842	*KIAA0226*	*P070 probe for 3q. Located between the telomere and the microdeletion syndrome region.*		

- The P036 Subtelomeres Mix 1 probe for the *BDH1* gene is also located in the commonly deleted region. However, the *KIAA0226* probe in P070 Subtelomeres Mix 2B is not, as it is located between the commonly deleted region and the telomere.
- A microduplication of the 3q29 region (OMIM 611936) has been described once (Lisi et al. 2008). Phenotype includes mild to moderate ID and minor dysmorphic features.
- More information on 3q29 microdeletion syndrome can be found in OMIM 609425. Phenotype can vary widely between patients and may include mild to moderate ID, dysmorphic features, autism, chest wall deformities, and ataxia.

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

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	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*		*1308 kb*
232 «	04190-L05920	LETM1	04-001.81	CCTGTGTACACA-TCCTCCAGAGGC	52 kb
454	10633-L14379	WHSC1	04-001.87	GTGGGCATTTAT-TTTCCCTTAATG	

- The most frequent cause is a terminal deletion of 4p16.3 that can also be detected by the telomeric probemixes P036 Subtelomeres Mix 1 and P070 Subtelomeres Mix 2B.
- 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 DD and ID, seizures and skeletal anomalies.

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

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	02791-L02233	*CCDC127*	*P070 probe for 5p*		*109 kb*
	01723-L1327	*PDCD6*	*P036 probe for 5p*		*968 kb*
445	03761-L22817	TERT	05-001.34	TCTTTCTTTTAT-GTCACGGAGACC	8155 kb
283	14265-L22700	SEMA5A	05-009.49	ACTTGGGCTGGA-GTGCCCACGTGG	

- 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 Subtelomeres Mix 1 and P070 Subtelomeres Mix 2B. Interstitial deletions have also been described (Zhang et al. 2005). Some interstitial deletions will not be detected by the two 5p15 probes in this P245 Microdeletion Syndromes-1A probemix.
- More information on Cri-du-Chat syndrome can be found in OMIM 123450. Clinical features include severe psychomotor retardation, ID and the characteristic cat-like cry.

Table 2h. Sotos syndrome, 5q35.3

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
154	02595-L23366	NSD1	05-176.62	ACCCACCCACTG-TTATGCAGAACA	32 kb
462	02600-L15191	NSD1	05-176.65	GGAAAGACTGTT-TGCAAATGTGGA	

- More probes for the *NSD1* gene are present in the P026 Sotos 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 Subtelomeres Mix 1 and P070 Subtelomeres Mix 2B 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 ID and delayed motor development.

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

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
364	01336-L00878	ELN	07-073.11	TTTCCCGGCTTT-GGTGTCGGAGTC	12 kb
315 «	16349-L22813	ELN	07-073.12	ACCTCATCAACG-TTGGTGCTACTG	

- More probes in the Williams-Beuren syndrome (WBS) region are present in SALSA MLPA Probemix P029 WBS. 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, ID, and distinctive facial features.

Table 2j. Langer-Giedion syndrome, 8q24.11-q24.13

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
401 «	03081-L07411	TRPS1	08-116.75	CTCTTTTTTGGT-GCTGCTGGTTTC	2168 kb
429	15322-L17698	EXT1	08-118.92	GGTGATAATGTT-AAACCCACTTAA	

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

Table 2k. 9q22.3 microdeletion syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
436	04460-L22816	FANCC	09-096.90	GATAACTCACGA-GATCATTGGCTT	377 kb
323	03702-L22814	PTCH1	09-097.28	GTTAATGACTCC-CAAGCAAATGTA	

- An interstitial 9q22.3 microdeletion syndrome has been described by Redon et al. 2006. Clinical phenotype includes ID, overgrowth and trigonocephaly. Please note that their patients had a 6 Mb deletion.
- Microduplications of 9q22.3, spanning at least the *PTCH1* gene, have been described (Izumi et al. 2011; Weise et al. 2012).
- The clinical spectrum of the 9q22.3 microdeletion is variable and the clinical findings depend on the size of the microdeletion. All reported 9q22.3 microdeletions include the *PTCH1* gene, which is involved in Gorlin syndrome (nevoid basal cell carcinoma syndrome); therefore, all individuals with 9q22.3 microdeletion have the clinical findings of this well-described disorder. Additional characteristics in 9q22.3 patients include seizures and DD and/or ID.

Table 2l. DiGeorge syndrome (DGS)/velocardiofacial syndrome complex (VCFS) 2, 10p13-p14

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
136	07632-L07317	GATA3	10-008.14	GAGCAACGCAAT-CTGACCGAGCAG	

- More information on DGS/VCFS 2 can be found in OMIM 601362. Phenotype includes similar features as described for 22q11.2 deletion syndrome (see Table 2t), however, phenotypes can vary widely between affected patients.
- More probes for the 10p14 region are present in the SALSA MLPA Probemix P250 22q11/DiGeorge.
- In addition to this 10p13-p14 region, deletion of the 17p terminal region can also cause a phenotype similar to 22q11.2 deletion syndrome. These 17p deletions should be detectable by P036 Subtelomeres Mix 1 and P070 Subtelomeres Mix 2B.

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

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	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"*		*1143 kb*

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
244	12178-L13826	SNRPN	15-022.63	ACCACCACCTGA-TGAAAGATACAC	138 kb
300 #	01318-L23196	SNRPN	15-022.76	GATTCCTCGCTA-CTCCAATATGGC	441 kb
166	10877-L11547	UBE3A	15-023.20	AGTGTTATTGGA-AGTGAGCCACCA	

- More probes for the Prader-Willi / Angelman region, including probes for the detection of methylation changes, are present in ME028 Prader-Wili/Angelman.
- The majority of the Prader-Willi and Angelman patients have a copy number change of the 15q11.2 region that should be detected by P245 Microdeletion Syndromes-1A. 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 probemix, but not with P245 Microdeletion Syndromes-1A.
- 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 2n. Witteveen-Kolk syndrome / 15q24 microdeletion syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
190	18316-L23369	SEMA7A	15-072.49	TACCCACAGAGA-CCTTCCAGGTGG	308 kb
331 ∫	06811-L22815	CYP1A1	15-072.80	GTCAACCTGAAT-AATAATTTCGGG	

- Probes for other genes located in the 15q24 microdeletion syndrome region are present in the SALSA MLPA Probemix P297 Microdeletion Syndromes-2.
- Please note that the *SIN3A* gene, which has been described as the critical gene in Witteveen-Kolk syndrome, is not covered by the probes in P245 Microdeletion Syndromes-1A. Point mutations in this gene, leading to Witteveen-Kolk syndrome, cannot be detected by P245 Microdeletion Syndromes-1A.
- More information on 15q24 microdeletion syndrome can be found in OMIM 613406. This syndrome has been termed Witteveen-Kolk syndrome (WITKOS) as described by Witteveen et al. (2016). Phenotype includes mild to severe ID, growth retardation, facial dysmorphisms, and failure to thrive.

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

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
172 «	03087-L02487	CREBBP	16-003.87	AGCAGGTGAAAA-TGGCTGAGAACT	

- More probes for the *CREBBP* gene are present in the SALSA MLPA Probemix P313 CREBBP.
- 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 this single probe, 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 ID.

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

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
142	04120-L03532	PAFAH1B1	17-002.51	TGTAGGCACTCT-ATAGATCAAGCT	2 kb
238 #	16348-L22830	PAFAH1B1	17-002.52	CCAGAAAAATAT-GCATTGAGTGGT	

- More probes for *PAFAH1B1* and other genes in the Miller-Dieker region are present in SALSA MLPA Probemix P061 Lissencephaly.
- 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 2q. Smith-Magenis syndrome / Potocki-Lupski syndrome, 17p11.2

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
471	11730-L15192	RAI1	17-017.57	CCAAGGATCTCA-TCTGGCCACCGC	264 kb

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
278	01452-L20745	DRC3	17-017.83	CGGATCTCCAAG-ATCGACTCCCTG	245 kb
307 «	01453-L22689	LLGL1	17-018.08	CAGCAGTCTGCA-TCTCTGGGAGAT	

- More probes for the Smith-Magenis region are present in SALSA MLPA Probemix P369 Smith-Magenis.
- 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, DD, cognitive impairment, behavioural abnormalities, and mild to moderate ID. PTLS is characterised by hypotonia, failure to thrive, ID, pervasive developmental disorders, and congenital anomalies.

Table 2r. *NF1* microdeletion syndrome, 17q11.2

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
339	02507-L22694	NF1	17-026.58	GGATCATGAAGA-ATTACTACGTAC	113 kb
260	11732-L13830	NF1	17-026.69	TCTTGTTGTCTT-TGGGTGTATTAG	

- More probes for the *NF1* gene are present in SALSA MLPA Probemix P081 NF1 mix 1 and SALSA MLPA Probemix P082 NF1 mix 2. More probes for other genes in this area are present in SALSA MLPA Probemix P122 NF1-area.
- Approximately 5-20% of all neurofibromatosis type 1 patients carry a heterozygous deletion of approximately 1.4 Mb that includes the *NF1* gene. Compared to patients with a point mutation, patients with a deletion of the *NF1* gene often present with a more severe phenotype.
- More information on *NF1* microdeletion syndrome can be found in OMIM 613675. Information on neurofibromatosis type 1 can be found on https://www.ncbi.nlm.nih.gov/books/NBK1109/ and in OMIM 162200. The *NF1* microdeletion results in a phenotype that often includes facial dysmorphism, ID, DD, and excessive early-onset neurofibromas.

Table 2s. Koolen-de Vries syndrome / 17q21.31 microduplication syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
272	08365-L22699	MAPT	17-041.46	GTCGCCAGTGGT-GTCTGGGGACAC	43 kb
346	18172-L22729	KANSL1	17-041.50	CCGCTTCTTACA-GCTCAGTACAGG	

- More probes for the *MAPT* gene and other genes in the 17q21.31 region are present in SALSA MLPA Probemix P275 MAPT-GRN.
- The cause of Koolen-de Vries syndrome (KDVS) is a deletion of the *KANSL1* gene as described by Koolen et al. (2012). Patients with a duplication of the same region differ phenotypically from KDVS patients (Kirchoff et al. 2007; Grisart et al. 2009).
- More information on KDVS can be found on http://www.ncbi.nlm.nih.gov/books/NBK24676/ and in OMIM 610443. The 17q21.31 microduplication syndrome is described in OMIM 613533.

Table 2t. 22q11.2 deletion syndrome / 22q11.2 duplication syndrome / distal 22q11.2 deletion syndrome

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	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*		*1285 kb*
196	01218-L06270	CLDN5, region AB	22-017.89	TTCGCCAACATT-GTCGTCCGCGAG	200 kb
208 « ±	05464-L15184	GP1BB, region AB	22-018.09	CACAACCGAGCT-GGTGCTGACCGG	1474 kb
373	16748-L19368	SNAP29, region CD	22-019.57	GTATCCACTTAC-CTGTATCATCCA	814 kb
220	07530-L22697	PPIL2; distal 22q11	22-020.38	GAAGAGCCCTCA-ACCAGTGCCACT	1354 kb
265 «	08484-L22698	RTDR1; distal 22q11	22-021.73	GGTGTGTCATTT-TGACGTCATCCC	

- More probes for the 22q11 DiGeorge region are present in P250 22q11/DiGeorge.
- 22q11 deletions related to the eponymous syndrome 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 P036 Subtelomeres Mix 1 and P070 Subtelomeres Mix 2B as well as P250 22q11/DiGeorge, but not by the probes in P245 Microdeletion Syndromes-1A.
- More information on 22q11.2 deletion 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 22q11.2 deletion syndrome, including congenital heart disease, palatal abnormalities, characteristic facial features, learning difficulties, immune deficiency, and hypocalcaemia.
- The chromosome 22q11.2 distal deletion syndrome (OMIM 611867) has been described by e.g. Ben-Shachar et al. (2008). This syndrome frequently results in DD, growth delay and mild skeletal abnormalities.
- Several distal 22q11 duplications have been described (reviewed by Pinchefsky et al. 2017). These distal 22q11 duplications can be variable in size and may be detected by the 220 nt and/or 265 nt probes in P245 Microdeletion Syndromes-1A.

Table 2u. Phelan-McDermid syndrome, 22q13

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
	02707-L00661	*ARSA*	*P070 probe for 22q13*		*48 kb*
252 «	12031-L13828	SHANK3	22-049.46	AAGCGGCGAGTT-TATGCCCAGAAC	91 kb
382 #	06734-L05558	RABL2B	22-049.55	AATACACAAGCC-GTAAAATCGAGT	

- More probes in the Phelan-McDermid region are present in SALSA MLPA Probemix P188 22q13.
- The *SHANK3* gene is suspected to be responsible for at least part of the Phelan-McDermid syndrome phenotype. The *RABL2B* probe in P036 Subtelomeres Mix 1 is located between *SHANK3* and the 22q telomere. The *RABL2B* probes in P245 Microdeletion Syndromes-1A and P036 Subtelomeres Mix 1 detect almost the same sequences.
- 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 DD, normal to accelerated growth, absent to severely delayed speech, autistic behaviour, and minor dysmorphic features. Most individuals have moderate to profound ID.

Table 2v. X chromosome copy number changes

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18 [b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
292	01411-L23371	DMD	X-031.56	AAACTCATAGAT-TACTGCAACAGT	

- This DMD probe is intended to be used to distinguish *MECP2* duplications from X chromosome copy number alterations. This probe must not be used to detect defects in the *DMD* gene.

Table 2w. Rett syndrome / *MECP2* duplication syndrome, Xq28

Length (nt)	SALSA MLPA probe	Gene	MV36/hg18[b]	Partial sequence[a] (24 nt adjacent to ligation site)	Distance to next probe
202 «	03409-L16570	Exon 1	X-153.02	CATTAATCCTTA-ACATTCAAATTC	65 kb
184 «	15319-L17592	Exon 3	X-152.95	ACTTGTTCTGCA-GACTGGCATGTT	2 kb
148 «	09310-L13824	Exon 4	X-152.95	TTTCATCCTCCA-TGCCAAGGCCAA	

- More probes for the *MECP2* gene are present in SALSA MLPA Probemix P015 MECP2. More probes in the Xq28 region are present in SALSA MLPA Probemix P049 SLC6A8 – ABCD1.
- Approximately 8% of Rett syndrome patients have a deletion involving the *MECP2* gene. A duplication of this gene appears to be a relatively frequent cause of ID, leading to the *MECP2* duplication syndrome (also known as Lubs X-linked mental retardation syndrome or MRXSL).
- More information on Rett syndrome can be found on http://www.ncbi.nlm.nih.gov/books/NBK1497/ and in OMIM 312750.
- More information on *MECP2* duplication syndrome can be found on https://www.ncbi.nlm.nih.gov/books/NBK1284/ and in OMIM 300260.
- Rett syndrome almost exclusively affects females and clinical features include small hands and feet, deceleration of the rate of head, and repetitive stereotyped hand movements. Gastrointestinal disorders, seizures and scoliosis occur in most patients. The *MECP2* duplication syndrome largely affects young males. Symptoms include infantile hypotonia, delayed psychomotor development leading to severe ID, impaired speech, epilepsy, recurrent infections, and early death.

[a] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.
[b]'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.

« Probe located within, 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.
± SNV rs749912012 could influence the 208 nt probe signal. SNV rs747391156 could influence the 226 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.
∫ Probe is very sensitive to pipetting mistakes.

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

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

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

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P245 product history	
Version	*Modification*
B1	Three new microdeletion syndrome regions have been included (2q23, 2q33, distal 22q11) and one region has been removed (WAGR syndrome). Several of the other probes have been replaced.
A2	The 108 nt Y probe has been removed and new control fragments at 100 and 105 nt have been added.
A	First release.

Implemented changes in the product description

Version B1-13 – 22 June 2026 (05P)
-Product description adapted to a new template.
-Throughout the document, the name of SALSA MLPA Probemix P250 22q11/DiGeorge was updated (previously P250 DiGeorge).
-Under tables 2l and 2t, the nomenclature has been updated: DiGeorge-2 has been replaced with DiGeorge syndrome (DGS)/velocardiofacial syndrome complex (VCFS) 2; DiGeorge syndrome has been replaced with 22q11.2 deletion syndrome (with DiGeorge only being used to describe the specific region in 22q11.2). These updates have been applied to all instances in which the old names of these syndromes were used.
-Under table 2t, it has been specified that SALSA MLPA Probemix P250 22q11/DiGeorge also has targets for the Cat Eye Syndrome region.
-Full product names have been added for probemixes when mentioned for the first time.
-The term *mental retardation* has been replaced with *intellectual disability* throughout the document.
-Abbreviations ID and DD used throughout the document for intellectual disability and developmental delay, respectively.

Version B1-12 – 4 September 2025 (04P)
- Product is registered for IVD use in Morocco.

Version B1-11 – 12 April 2023 (04P)
- Product is no longer registered for IVD use in Morocco.

Version B1-10 – 23 February 2023 (04P)
- New warning added to *Precautions and warnings* recommending to analyse male and female samples separately.
- 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.*
- Minor corrections were made to Table 1 and 2: length of control fragments was corrected (64-118 nt) in Table 1; several chromosomal bands were updated for consistency in Table 1; for applicable syndromes *microduplication* was added in last column of Table 1; title of Table 2 corrected; background information in notes below Tables 2a-w was updated.
- Section *Related SALSA MLPA probemixes* updated for P297.
- Curated the section *Selected publications*.
- Various minor textual or layout changes.

Version B1-09 – 07 December 2022 (04P) - Information about the 118 nt Y-chromosome control fragment added to section 'Probemix content'.

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