Product Description

SALSA® MLPA® Probemix P036-E3 Subtelomeres Mix 1

To be used with the MLPA General Protocol.

Version E3
For complete product history see page 11.

Catalogue numbers:

- **P036-025R:** SALSA MLPA Probemix P036 Subtelomeres Mix 1, 25 reactions.
- **P036-050R:** SALSA MLPA Probemix P036 Subtelomeres Mix 1, 50 reactions.
- **P036-100R:** SALSA MLPA Probemix P036 Subtelomeres Mix 1, 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 P036 Subtelomeres Mix 1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletion(s) or duplication(s) in subtelomeric regions in genomic DNA isolated from human peripheral whole blood specimens, 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, fetal blood or products of conception free from maternal contamination. P036 Subtelomeres Mix 1 is intended to confirm a potential cause for and clinical diagnosis of intellectual disability, developmental delay, congenital abnormalities and/or pregnancy loss and for molecular genetic testing of at-risk family members.

It is recommended that results of P036 Subtelomeres Mix 1 are confirmed with P070 Subtelomeres Mix 2B. Copy number variations (CNVs) detected with one or both probemixes should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method.

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.
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
In the general population, the prevalence of intellectual disability and developmental delay is estimated at 1-3% and the prevalence of congenital anomalies at 2-3% (Castells-Sarret et al. 2017). Clinical signs can include dysmorphic features, language impairment, seizures, learning disabilities, behavioural disturbances and

autism spectrum disorders. It has been recognized that worldwide the genetic etiology of individuals with non-syndromic intellectual disability remains undetermined in the majority of cases. Determining the etiology of intellectual disability and developmental delay is important and useful for pediatric neurologists, geneticists, pediatricians, and patients' families because it allows assessment of recurrence risk, appropriate genetic counselling, and focus on treatment options and prognosis.

Aberrant copy numbers of subtelomeric regions, e.g. due to an unbalanced translocation, are a frequent cause of intellectual disability, developmental delay and/or congenital abnormalities lacking distinct syndromic features. Subtelomeric regions are more likely to be involved in copy number changes as these are less likely to be lethal due to the low amount of critical genes located in these regions. The differences in size and breakpoint location of chromosomal CNVs make the phenotype highly variable among patients, ranging from a non-viable fetus to a phenotypically normal individual. Chromosomal anomalies of the fetus are found in almost half of the miscarriages and are the most common reason for pregnancy loss (Bernatowicz et al. 2019). More information can be found on Decipher (http://decipher.sanger.ac.uk/) and in the references listed at the end of this product description.

Probemix content

The SALSA MLPA Probemix P036-E3 Subtelomeres Mix 1 contains 46 MLPA probes with amplification products between 130 and 486 nucleotides (nt). This includes 41 probe(s) for the subtelomeric regions, no probes are present for the subtelomeric regions on the short p-arm of the 5 acrocentric chromosomes (13, 14, 15, 21 and 22). For these, an extra probe is included detecting the q-arm, close to the centromere. The subtelomeric probes for the X and Y chromosome are identical as they detect sequences in the pseudo-autosomal regions (PAR1 and PAR2) which are identical in chromosome X and Y. More information is present in Table 1 of this document. Complete probe sequences are available online (www.mrcholland.com).

This probemix contains ten quality control fragments generating amplification products between 64 and 121 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)
105-121	Y-fragments (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 human peripheral whole blood specimens, buccal swab, (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, fetal blood, or products of conception free from maternal contamination,

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
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 without intellectual disability, developmental delay and/or congenital abnormalities. 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 samples from the Coriell Institute listed in the table below have been tested with this P036-E3 probemix at MRC Holland and can be used as positive control samples to detect CNVs in subtelomeric regions, see table below for more detailed information. The quality of cell lines can change; therefore samples should be validated before use.

Coriell sample ID	Chromosomal position of copy number alteration*	Probe targets affected	Copy number alteration
NA06047	17p	*RPH3AL*	Heterozygous deletion
NA22991	1p	*TNFRSF4*	Heterozygous deletion
NA14131	5p	*PDCD6*	Heterozygous deletion
NA00501	2p and 4q	Upstream of *ACP1* and *TRIML2*	Heterozygous deletion of 2p Heterozygous duplication of 4q

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P036-E3 Subtelomeres Mix 1 probemix.

Performance characteristics
The exact performance characteristics cannot easily be determined due to the difficulty in obtaining sufficient DNA samples containing deletions or duplications for all probes. Ahn et al. (2007) determined 95% confidence intervals for positive and negative predictive accuracies to be 0.951-0.996 and 0.9996-1 respectively in their laboratory. In general, each positive result needs to be confirmed and further studied.

In postnatal samples from patients with non-syndromic intellectual disability and/or developmental delay, the P036 and P070 subtelomere probemixes detect subtelomeric CNVs in approximately 3-6% of the cases. However, the diagnostic sensitivity depends highly on the inclusion criteria of the patients that were tested. When patients with non-syndromic intellectual disability, developmental delay and congenital abnormalities are included, CNVs are found in approximately 10% of the cases. For prenatal samples, the diagnostic sensitivity is 0.5-4% and the diagnostic sensitivity for products of conception (POC) is 18-62%.

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 allele copy number for all probes, including the probes for the pseudo-autosomal regions of the sex chromosomes, are 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication). The standard deviation of each individual probe over all the reference samples should be ≤0.10. When this criterium 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.

- 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.
- False results can be obtained if one or more peaks are off-scale. 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.
- Interpretation of abnormal copy number findings in subtelomeric regions is complicated. Subtelomeric copy number changes can also occur in unaffected individuals and the effect of a deletion or duplication will depend on the genes involved. A considerable number of abnormalities detected by a single probe may not be the cause of any phenotypic effect but can be due to a rare polymorphism or a copy number change which is also present in one of the parents. For some chromosome arms, even large subtelomeric deletions or duplications (>1 Mb) can be inherited without a clear phenotypic effect. For all abnormalities detected, we strongly recommend testing parents to determine whether the copy number aberration in the patient is de novo.
- 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.

P036 specific notes:

- The pathogenic phenotypes caused by subtelomeric deletions or duplications are in most cases autosomal dominant disorders. Copy number changes of a single chromosome can be pathogenic.

- Deletions can result in haploinsufficiency of one or more genes. Duplications can lead to distortion of gene expression or can result in haploinsufficiency of genes that are disrupted by the duplication breakpoints. Analysis of parental samples may be necessary for correct interpretation of results.
- For each subtelomeric region, the copy number of only a single small (approximately 70 nt) sequence is determined. **These sequences have been selected on the basis of their proximity to the telomere and are usually located in genes that have not been directly implicated in intellectual disability, developmental delay and/or congenital abnormalities.** For many chromosomes, it is not known what minimum size is required for a subtelomeric deletion/duplication to have pathogenic effects.
- Most probes in the P036 and P070 probemixes target well-characterised genes at short distance of the telomere. An exception is made for the p-arms of chromosomes 13, 14, 15, 21 and 22 as these are covered by over 10 Mb of repeat sequences. Here, the "p" probes recognise one of the first genes on the q arm, close to centromere.

Limitations of the procedure

- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- For use on (un)cultured amniocytes and (un)cultured chorionic villi, contamination of the sample with maternal DNA may lead to wrong conclusions.
- For use on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and fetus have been described due to maternal contamination, postzygotic nondisjunction, postzygotic isochromosome formation, mosaic situations, and complications in DNA sampling in twin pregnancies (Van den Berg et al. 2006).

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.

False negative results can occur given that each chromosomal arm is covered by a single probe; whereas false positive results may be due to polymorphisms or experimental variation. Given the high chance of false positive/negative results, it is recommended to use both MLPA probemix P036 and MLPA probemix P070 for initial testing of each DNA sample. **All P070 probes differ from P036 probes.** Copy number changes detected by P036 **and** P070 may still be without clinical significance. In case of positive results, it is therefore strongly recommended to also test the parents. **In case P036 and P070 give conflicting results, follow-up studies are needed, see Figure 1.**

To confirm and delineate the extent of a subtelomeric deletion/duplication, MLPA follow-up probemixes containing multiple probes for a particular subtelomeric region are available, see related probemixes and/or www.mrcholland.com. Confirmation may also be done by FISH, array CGH or NGS. Confirmation of results may be difficult: deletions/duplications can be interstitial and may not extend to the telomere. P036 and P070 probes may detect sequences which are located quite far from each other or from the targets of commercially available FISH probes.

In case of suspected aneuploidy in which probes of the q-arm and p-arm (or q-cen for acrocentric chromosomes 13, 14, 15, 21 and 22) of a specific chromosome are affected in the same way, confirmation

should be done by alternative methods, or follow-up MLPA probemix P095 can be used to detect aneuploidies of chromosomes 13, 18, 21, X and Y.

Database of Genomic variants and Phenotype
http://decipher.sanger.ac.uk/. We strongly encourage users to deposit positive results in the Decipher database.

Please report false positive results due to SNVs and other unusual results to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P036-E3 Subtelomeres Mix 1

Length (nt)	SALSA MLPA probe	Gene detected	Chromosomal position (hg18)	Location (hg18) in kb
64-121	Control fragments – see table in probemix content section for more information			
130 «	02269-L01761	TNFRSF4	1p	01-001,137
136 ±	02274-L08758	Upstream of ACP1	2p	02-000,252
144	01721-L01329	CHL1	3p	03-000,345
152	02005-L02047	PIGG (FLJ20265)	4p	04-000,505
160	01723-L01327	PDCD6	5p	05-000,368
166	01724-L02048	IRF4	6p	06-000,338
172 «	02275-L02049	ADAP1 (CENTA1)	7p	07-000,926
180	02397-L01845	FBXO25	8p	08-000,403
186	01727-L02050	DMRT1	9p	09-000,837
193	02277-L01768	DIP2C (KIAA0934)	10p	10-000,477
202	03315-L02733	RIC8A (RIC-8)	11p	11-000,200
208 « ±	02276-L01767	SLC6A12	12p	12-000,170
219 + «	02399-L01847	PSPC1	13q-cen	13-019,245 (Acrocentric chromosome)
227 +	01732-L01318	CCNB1IP1 (HEI10)	14q-cen	14-019,864 (Acrocentric chromosome)
235 + #	07291-L08858	MKRN3	15q-cen	15-021,363 (Acrocentric chromosome)
242 ±	01734-L01316	POLR3K	16p	16-000,043
250	01735-L01315	RPH3AL	17p	17-000,169
258	01736-L02051	USP14	18p	18-000,187
265 «	01737-L01313	CDC34	19p	19-000,492
274	02396-L01844	SOX12	20p	20-000,255
283 +	18223-L23852	RBM11	21q-cen	21-014,510 (Acrocentric chromosome)
289 +	01740-L01310	BID	22q-cen	22-016,607 (Acrocentric chromosome)
298	01148-L01331	SHOX	Xp/Yp (PAR1)	X/Y-000,522 (PAR1 region)
307	02392-L02149	SH3BP5L (KIAA1720)	1q	01-247,075 (0.2 Mb from telomere)
313	01742-L01308	CAPN10	2q	02-241,179 (1.8 Mb from telomere)
322	02013-L02052	BDH1	3q	03-198,758 (0.7 Mb from telomere)
330	12050-L11446	TRIML2	4q	04-189,263 (2.0 Mb from telomere)
337 #	03319-L02737	GNB2L1	5q	05-180,598 (0.3 Mb from telomere)
346	01746-L01304	PSMB1	6q	06-170,688 (0.2 Mb from telomere)
355 ±	01747-L01303	VIPR2	7q	07-158,595 (0.2 Mb from telomere)
362	01748-L01302	ZC3H3 (KIAA0150)	8q	08-144,692 (1.6 Mb from telomere)
372	08205-L08170	EHMT1	9q	09-139,830 (0.4 Mb from telomere)
379 « ±	09142-L09953	PAOX (PAO)	10q	10-135,045 (0.3 Mb from telomere)
387	01751-L01299	NCAPD3 (KIAA0056)	11q	11-133,596 (0.9 Mb from telomere)
395	02687-L02154	ZNF10	12q	12-132,244 (0.1 Mb from telomere)
402 ±	01753-L01297	F7	13q	13-112,818 (1.3 Mb from telomere)
411 «	02778-L02201	MTA1	14q	14-104,996 (1.4 Mb from telomere)

Length (nt)	SALSA MLPA probe	Gene detected	Chromosomal position (hg18)	Location (hg18) in kb
418 «	01755-L01295	ALDH1A3	15q	15-099,265 (1.1 Mb from telomere)
426	03201-L02669	GAS8 (GAS11)	16q	16-088,630 (0.2 Mb from telomere)
434	01757-L01293	TBCD	17q	17-078,452 (0.3 Mb from telomere)
440	01758-L01292	RBFA (C18orf22)	18q	18-075,900 (0.2 Mb from telomere)
450 «	09143-L10626	CHMP2A (BC-2)	19q	19-063,755 (0.1 Mb from telomere)
457 ±	02688-L02884	OPRL1	20q	20-062,195 (0.2 Mb from telomere)
467	02586-L02059	PRMT2 (HMT1)	21q	21-046,888 (0.1 Mb from telomere)
477 #	01762-L08761	RABL2B	22q	22-049,553 (0.1 Mb from telomere)
486	01763-L02150	VAMP7 (SYBL1)	Xq/Yq (PAR2)	X-154,781 + Y-057,640 (PAR2; both 0.1 Mb from telomere)

± SNP rs72772902 and rs536439268 could influence the 136 probe; rs60220187 could influence the 208 probe; rs114777900 could influence the 242 probe; rs562399123 could influence the 355 probe; rs78367010 could influence the 379 probe; rs139304588 could influence the 402 probe; rs113943566 could influence the 457 probe. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
+ The 13, 14, 15, 21 & 22 q-cen probes target the q-arm close to the centromere (acrocentric chromosomes), as the p-arm of these chromosome do not contain well-characterised genes.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the table above.

Important notes on specific subtelomere regions
Several probes are located in regions that show copy number variation in healthy individuals, see http://dgv.tcag.ca/dgv/app/home. Therefore, we strongly recommend ALWAYS confirming results by a designated MLPA follow-up probemix or other suitable method.

Please inform us about any possible improvements or interesting findings: info@mrcholland.com.

- **1p**: The **P070** probe detects a sequence that was found to be duplicated in a healthy individual.
- **3p**: The **P036/P070** probes detect sequences that were found to be duplicated in healthy individuals. According to Dijkhuizen et al. (2006), defects in the more centromeric *CNTN4* and *CRBN* genes might be more important for the 3p syndrome than *CHL1* loss.
- **12p**: The **P036/P070** probes detect sequences that were found to be duplicated in healthy individuals.
- **15q-cen**: The **P036/P070** probes detect sequences that were found deleted in some Prader-Willi/Angelman syndrome (PWS/AS) patients. We recommend SALSA MLPA Probemix ME028 Prader-Willi/Angelman for further characterisation of the 15q11-13 region.
- **16p**: The **P036/P070** probes are variable. P036 and P070 probes target sequences that are 0.36 Mb separated and, therefore, might not always be able to confirm each other. The SALSA MLPA Probemix P140 HBA (IVD) contains multiple probes on the 16p subtelomere region and can be used to further characterise 16p deletions and duplications.
- **20p**: The **P036/P070** probes detect sequences that were found to be duplicated in healthy individuals.
- **3q**: The **P036** probe for 3q (*BDH1* gene) was found to be duplicated in a healthy parent by E. Reyniers (Medical Genetics, University of Antwerp), this was confirmed by FISH. The **P070** probe for 3q, located 200 kb closer to the telomere, did not show this duplication. Apparently, CNVs of the *BDH1* gene region can occur in healthy individuals. However, please note that a 3q29 microdeletion syndrome (due to an interstitial deletion) has been described (Willatt et al. 2005) and that the probe in the **P036** probemix detects a sequence within this commonly deleted region. The 3q probe in **P070** will not detect this microdeletion, as it detects a sequence located between the interstitial deletion and the telomere.
- **4q**: The **P036/P070** probes detect the complex 4q telomeric region in which very few genes are present. The *FRG1* gene is the only well-characterised gene in the terminal region of 4q, but most *FRG1*-specific

probes tested were found to be unreliable due to the presence of population-specific SNPs in *FRG1*. The 4q probe in **P036** is located at a larger distance from the telomere; we do not expect this to result in false-negatives as deletions of the telomeric 2-3Mb region of 4q are not associated with any phenotypic effects (Shao et al. 2008).

- **5q**: The **P036** probe for 5q was affected in one patient due to a polymorphism in the first nucleotide after the ligation site. The **P036/P070** probes for 5q detect sequences that have been found to be duplicated sporadically in healthy individuals.
- **12q**: The **P036/P070** probes detect sequences that were found to be duplicated in a healthy individual.
- **19q**: The **P036/P070** probes detect sequences that have variable copy numbers in healthy individuals.
- **22q**: The **P070** probe detects a sequence that was found to be deleted in healthy individuals. The sequence detected by the **P036** probe for 22q has only one mismatch with a related sequence on chromosome 2. This is sufficient to generate a chr. 22q specific MLPA signal. However, when this related region is to be sequenced, primer design is complicated as only 4 mismatches are present in the 350 nt region containing this probe sequence. The SALSA MLPA Probemix P188 22q13 (Research use only) contains many probes close to the 22q13 telomere and can be used to confirm or further characterise 22q CNVs.
- **X, Y**: The sex chromosome specific probes in **P036/P070** detect sequences located on both X and Y chromosome close to the telomeres (PAR region) and will thus detect the combined copy number of X and Y.

SALSA MLPA probemixes P036 Subtelomeres Mix 1 and P070 Subtelomeres Mix 2B

Probemixes *P036 Subtelomeres Mix 1* and *P070 Subtelomeres Mix 2B* both contain one probe for each subtelomere and can be used to detect most deletions/duplications in the subtelomeric regions. Using both probemixes together maximises the detection rate and reduces the risk of false positives due to polymorphisms affecting a single probe. We recommend following the strategy outlined in Figure 1.

When used correctly, screening with MLPA probemixes P036 and P070 will detect or exclude the presence of abnormal copy numbers of subtelomeric regions in the majority of samples. MLPA will however not detect inversions or balanced translocations. Interpretation of abnormal copy number findings in subtelomeric regions is complicated. Subtelomeric copy number changes also occur in healthy individuals and the effect of a deletion or duplication will depend on the genes involved. The Database of Genomic Variants can be useful to verify whether copy number changes occur in normal individuals: http://dgv.tcag.ca/dgv/app/home.

A considerable number of abnormalities detected by a single probe may not be the cause of any phenotypic effect but can be due to a rare polymorphism. When abnormalities are detected using P036/P070, these findings should always be confirmed by an MLPA probemix for a specific syndrome (see www.mrcholland.com) or another technique (e.g. FISH, array-CGH).

Finding the genetic cause of congenital abnormalities with MLPA probemix P036 and P070

The number of genes associated with intellectual disability and/or developmental delay is large. In some cases, particular phenotypic features suggest the involvement of a specific gene or chromosomal region. Numerous SALSA MLPA probemixes are available to find the cause of developmental delay with distinct syndromic features, such as RETT, Sotos and Prader-Willi/Angelman syndrome.

For individuals with (suspicion of) intellectual disability or developmental delay and/or congenital abnormalities lacking distinct syndromic features, the genetic cause is found only in a minority of cases. When using the P036 and P070 probemixes, we suggest following the strategy as outlined in Figure 1.

When **no abnormalities** are detected using P036/P070, the follow-up SALSA MLPA Probemix P245 Microdeletion Syndromes-1A (IVD) can be used for screening for distinct microdeletion syndromes (see P245 product description for details). Numerous other probemixes can be found online at www.mrcholland.com and several probemixes are highlighted below in the Related SALSA MLPA probemixes section.

Finding the genetic cause of pregnancy loss with MLPA

The genetic cause of pregnancy loss can be found using P036/P070 on POC samples. Approximately 50-60% of first trimester spontaneous miscarriages are caused by chromosomal abnormalities, of which the vast majority is aneuploid (Wu T et al., 2019). In case of suspected aneuploidy, follow up probemixes or alternative methods are needed to confirm the results. We suggest following the strategy as outlined in Figure 1 below.

Prenatal diagnosis with MLPA

Using P036 and P070 for prenatal testing is recommended when there is an increased risk for intellectual disability, e.g. in high-risk pregnancies or when there is a suspicion of abnormalities, e.g. after ultrasound findings. We suggest following the strategy as outlined in Figure 1.

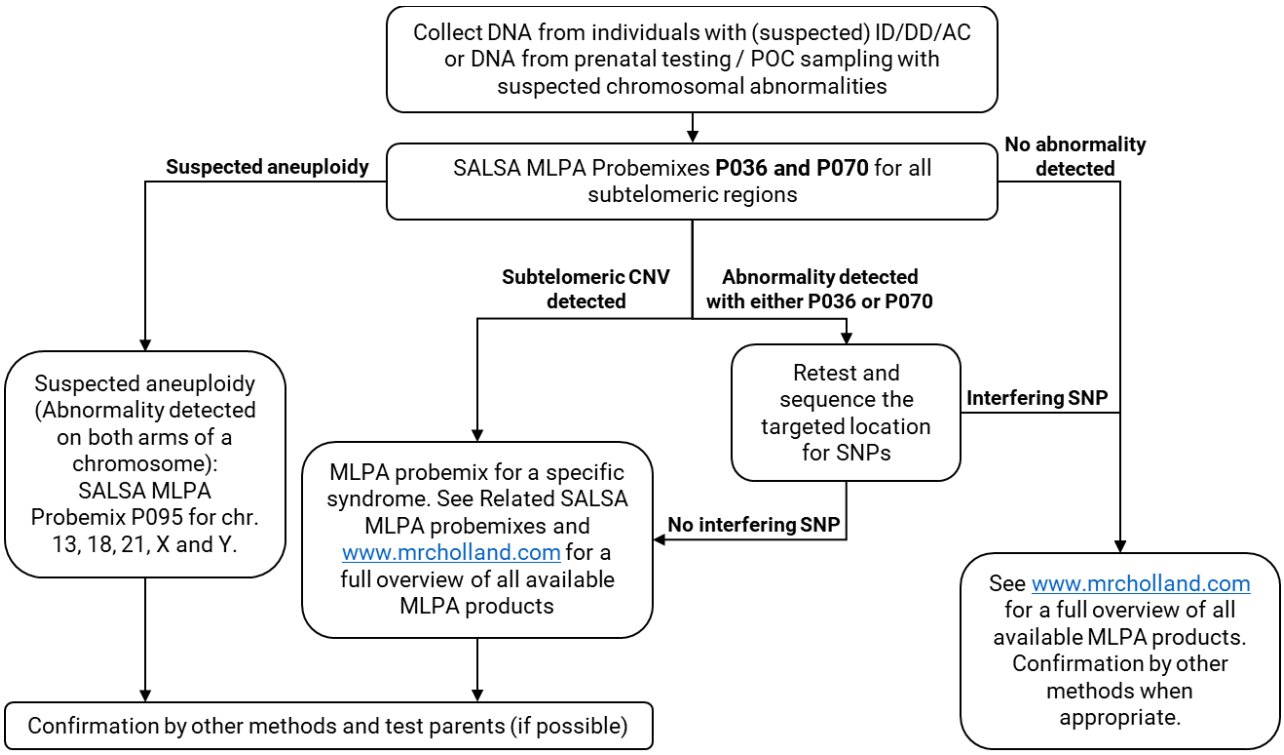

Figure 1: Flowchart suggesting how to test an individual with suspicion of ID/DD/CA lacking clear syndromic features and prenatal testing after higher risk for chromosomal abnormalities or POC testing. ID/DD/CA: intellectual disability / developmental delay / congenital abnormalities.

Related SALSA MLPA probemixes

P070 Subtelomeres Mix 2B	Contains one probe for each of the 41 subtelomeric regions and 5 probes near the centromeric regions of the five acrocentric chromosomes. All probes differ from the P036 probes. We strongly recommend using both P036 and P070 on each sample.
P245 Microdeletion Syndromes-1A	Probes for 23 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
P064 Microdeletion Syndromes-1B	Probes for 15 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
P297 Microdeletion Syndromes-2	Probes for 11 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
P106 MRX	Probes for several genes involved in X-linked mental retardation.
P095 Aneuploidy	Probes for chromosomes 13, 18, 21, X and Y to detect aneuploidy of these chromosomes.
P181 Centromere-1	Contains one probe for each of 41 different centromeric regions and an extra probe near the centromeric regions of each of the five acrocentric chromosomes, resulting in two probes for each chromosome.
P182 Centromere-2	Similar to P181 Centromere-1 but all probes are different from the P181 probes.
P147 1p36	Probes for the 1p36 subtelomeric region.
P140 HBA	Probes for the human alpha-globin (HBA) gene cluster and its regulatory region located on chromosome 16p13.3, as a potential cause for and clinical diagnosis of alpha-thalassaemia.

P188 22q13	Probes for the 22q12 and 22q13 chromosomal regions (research use only). A partial 22q13 deletion is associated with the Phelan-McDermid syndrome.
ME028 Prader-Willi/Angelman	Probes to detect an aberrant methylation of one or more sequences of the 15q11 chromosomal region, as well as copy number changes in this chromosomal region.

More probemixes are available for specific syndromes, including RETT(-like) syndrome, DiGeorge, Canavan, Lissencephaly, Williams syndrome and many more (see www.mrcholland.com).

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P036 product history	
Version	*Modification*
E3	The 118 nt chromosome Y probe has been elongated to 121 nt.
E2	DNA denaturation control fragments at 88 and 96 nt replaced (QDX2).
E1	Probes for 1p and 4q replaced.
D2	Chromosome X and Y specific control fragments at 100 and 105 nt added.
D	Probes for 1p and 4q replaced. DNA denaturation control fragments at 88 and 96 nt added.
C	Probes for 1p, 15p near the centromere, 4q and 9q replaced.
B	Probes for 11p, 4q, 5q, 10q, 12q, 14q, 16q, 19q, 20q and 21q replaced.
A	First release.

Implemented changes in the product description
Version E3-06 – 05 April 2023 (04P) - The research use only regulatory status of SALSA MLPA Probemix ME028 Prader-Willi/Angelman is removed throughout the P036/ P070 product descriptions as ME028 PWS/AS is a CE-marked IVD. - Selected publications section is updated. - Product no longer registered for IVD use in Morocco. - Various minor textual or layout changes. *Version E3-05 – 25 March 2021 (04P)* - Product description rewritten and adapted to a new template. - Intended purpose updated to include the recommendation of using P070 together with P036 and adapted to a new template. - Clinical background information rewritten. - Positive DNA sample information is updated. - Table 1 updated with new insights (e.g. SNPs and salt sensitive probes), probe location in kb and distance to telomere updated according to hg18. - Important notes paragraph updated with new insights, added Probemix P140 to confirm 16p. - P036/P070 paragraph rewritten and expanded to include prenatal and POC samples. - Figure 1 updated and expanded to include prenatal and POC samples. - SALSA MLPA probemixes P095, P297, P140, P188 and ME028 added to the related probemixes. - The sections References and Selected publications are updated. - UK has been added to the list of countries in Europe that accept the CE mark. *Version E3-04 – 17 June 2020 (02P)* - Israel added as country with IVD status. *Version E3-03 – 05 June 2018 (02P)* - Product description adapted to a new template. - Information regarding probemix P069 and the telomere follow-up probemixes has been removed. - Warning added to table 1 for probes relying on its specificity on a single nucleotide difference between target and related gene or pseudogene. - IVD use now includes Morocco.

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IVD	EUROPE* CE ISRAEL
RUO	ALL OTHER COUNTRIES

*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.