Product Description SALSA® MLPA® Probemix P106-D1 X-linked ID

To be used with the MLPA General Protocol.

Version D1. As compared to version C1, two probes have been replaced, three probes have been changed in length not in sequence detected. For complete product history see page 11.

Catalogue numbers:
- **P106-025R:** SALSA MLPA Probemix P106 X-linked ID, 25 reactions.
- **P106-050R:** SALSA MLPA Probemix P106 X-linked ID, 50 reactions.
- **P106-100R:** SALSA MLPA Probemix P106 X-linked ID, 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.mlpa.com).

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

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

General information: The SALSA MLPA Probemix P106 X-linked ID is a **research use only (RUO)** assay for the detection of deletions or duplications in several genes on the X chromosome, which are associated with X-linked intellectual disability (XLID).

Intellectual disability (ID) – used to be known as mental retardation (MR) – is estimated to occur in about 2-3% of the population. Among intellectually disabled patients, an excess of males over females has long been noted, which is usually explained by the presence of many genes responsible for ID on the X chromosome (Ropers and Hamel 2005).

XLID is usually divided into a syndromic and a non-syndromic form. In syndromic forms (S-XLID), ID is present in association with a specific pattern of physical, neurological, and/or metabolic abnormalities. The term non-specific or non-syndromic XLID (NS-XLID) was introduced to indicate a condition segregating in an X-linked manner in which male patients have no consistent phenotypic manifestations other than ID. Many different genes responsible for both forms of XLID have been identified (Ropers and Hamel 2005).

This P106 X-linked ID MLPA probemix can be used to detect copy number changes of several genes on the X chromosome that have been implicated in XLID: *RPS6KA3, ARX, IL1RAPL1, TSPAN7, PQBP1, HUWE1, OPHN1, ACSL4, PAK3, DCX, AGTR2, ARHGEF6, FMR1, AFF2, SLC6A8* and *GDI1*. For all genes, probes are present for only some of the exons.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering:
The exon numbering used in this P106-D1 X-linked ID product description is the exon numbering from the RefSeq transcripts:

- NM_004586.3, which is identical to the NG_007488.1 sequence (*RPS6KA3*)
- NM_139058.3, which is identical to the NG_008281.1 sequence (*ARX*)
- NM_014271.4, which is identical to the NG_008292.2 sequence (*IL1RAPL1*)
- NM_004615.3, which is identical to the NG_009160.1 sequence (*TSPAN7*)
- NM_005710.2, which is identical to the NG_015967.1 sequence (*PQBP1)*
- NM_031407.7, which is identical to the NG_016261.2 sequence (*HUWE1*)
- NM_002547.3, which is identical to the NG_008960.1 sequence (*OPHN1*)
- NM_022977.2, which is identical to the NG_008053.1 sequence (*ACSL4*)
- NM_002578.5, which is identical to the NG_008288.2 sequence (*PAK3)*
- NM_178152.3, which is identical to the NG_011750.1 sequence (*DCX)*
- NM_000686.5, which is identical to the NG_016326.1 sequence (*AGTR2*)
- NM_004840.3, which is identical to the NG_008873.1 sequence (*ARHGEF6*)
- NM_002024.5, which is identical to the LRG_762 sequence (*FMR1)*
- NM_002025.4, which is identical to the NG_016313.2 sequence (*AFF2)*
- NM_005629.4, which is identical to the NG_012016.2 sequence (*SLC6A8)*
- NM_001493.3, which is identical to the NG_008954.1 sequence (*GDI1)*

The exon numbering and NM-sequences used have been retrieved on 09/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P106-D1 X-linked ID contains 46 MLPA probes with amplification products between 130 and 481 nucleotides (nt). The probes detect sequences in the 16 genes described above. Complete probe sequences are available online (www.mlpa.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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105-121	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.mlpa.com).

MLPA technique validation: Internal validation of the MLPA technique using 16 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 handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from

families without a history of X-linked intellectual disability. It is recommended 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.

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) has a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

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

Secondly, inter-sample normalisation can be achieved by dividing the intra-normalised probe ratio in a sample by the average intra-normalised probe ratio of all reference samples.

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

Interpretation of results: The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results:

Copy Number status: Male samples	Dosage quotient
Normal	0.80 < DQ < 1.20
Deletion	DQ = 0
Duplication	1.65 < DQ < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region, especially in or near the *FMR1* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.

- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in most of the genes targeted by this P106 X-linked ID probemix are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P106 X-linked ID.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

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

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

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

Please report false positive results due to SNPs and unusual results (e.g., a duplication of *IL1RAPL1* exons 1 and 3 but not exon 2) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P106-D1 X-linked ID

Length (nt)	Chromosomal position (hg18)	Gene detected	SALSA MLPA probe	Location (hg18) in kb
64-121	Control fragments – see table in probemix content section for more information			
130	Xq23	*AGTR2*	13917-L02320	X-115,218
136 «	Xq28	*GDI1*	16875-L19669	X-153,319
142	Xq27.3	*FMR1*	02928-L03720	X-146,835
148 «	Xq22.3	*ACSL4*	02935-L02326	X-108,798
154 «	Xq28	*AFF2*	03511-L04202	X-147,390
160 «	Xp11.4	*TSPAN7*	02903-L02297	X-038,306
166 «	Xq27.3	*FMR1*	02927-L03721	X-146,822
172	Xq26.3	*ARHGEF6*	16857-L19651	X-135,617
178	Xp22.12	*RPS6KA3*	02907-L02301	X-020,084
184	Xp11.22	*HUWE1*	13919-L15456	X-053,598
188 * « #	Xq28	*SLC6A8*	22782-L32127	X-152,614
195 «	Xp21.3	*ARX*	18790-L24221	X-024,941
202	Xq26.3	*ARHGEF6*	02902-L04460	X-135,585
208 « #	Xq28	*SLC6A8*	01871-L15827	X-152,610
215	Xq23	*DCX*	04123-L15828	X-110,531
222 «	Xp21.3	*ARX*	02898-L04200	X-024,935
229 «	Xp21.3	*ARX*	13669-L15822	X-024,944
235	Xp21.3	*IL1RAPL1*	02922-L23556	X-029,211
241	Xq28	*AFF2*	03516-L15823	X-147,727
248 «	Xq22.3	*ACSL4*	03512-L23557	X-108,863
256	Xp11.22	*HUWE1*	13920-L23672	X-053,691
263	Xq28	*AFF2*	02933-L23673	X-147,877
268 ~	Xp11.4	*TSPAN7*	02904-L23558	X-038,420
275	Xq22.3	*DCX*	04124-L03481	X-110,463
283	Xq28	*AFF2*	00493-L00066	X-147,551
292	Xp21.3	*IL1RAPL1*	02920-L02314	X-028,516
301 *	Xq22.3	*ACSL4*	22783-L32128	X-108,774
309 ¥	Xp11.23	*PQBP1*	22016-L02878	X-048,641
319	Xq23	*DCX*	04121-L08390	X-110,541
328	Xp21.3	*IL1RAPL1*	02921-L02315	X-028,717
337	Xq28	*AFF2*	02932-L02323	X-147,845
343 ¥	Xq26.3	*ARHGEF6*	22017-L02293	X-135,691
355	Xq23	*AGTR2*	02925-L02319	X-115,216
364	Xp22.12	*RPS6KA3*	02906-L02300	X-020,137
371	Xq12	*OPHN1*	02912-L02306	X-067,570
378 «	Xq28	*GDI1*	16874-L23559	X-153,323
385	Xq22.3	*PAK3*	02908-L03178	X-110,253
392 ¥	Xp11.23	*PQBP1*	22856-L32371	X-048,644
400	Xq22.3	*PAK3*	03521-L02304	X-110,346
409 #	Xq12	*OPHN1*	02913-L23560	X-067,436
418	Xq22.3	*PAK3*	02909-L02303	X-110,293
427	Xp21.2	*IL1RAPL1*	02923-L23561	X-029,596
436	Xq12	*OPHN1*	02914-L02308	X-067,334
443	Xq26.3	*ARHGEF6*	16856-L19650	X-135,655
472	Xq12	*OPHN1*	02915-L02309	X-067,201
481	Xq22.3	*PAK3*	02911-L02305	X-110,350

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
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.
~ More variable. This probe has been reported to be deleted/duplicated in healthy individuals (various reports).

Table 2. P106-D1 probes arranged according to chromosomal location

Table 2a. *RPS6KA3* gene, Xp22.12

Length (nt)	SALSA MLPA probe	RPS6KA3 Exon[a]	Ligation site NM_004586.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2498-2500 (exon 22)*		
178	02907-L02301	Exon 21	2282-2283	ATCAGAGACTGA-CTGCTGCTCTTG	53.1 kb
364	02906-L02300	Exon 3	457-458	AAGGAAGGACAT-GAAAAGGCAGAT	4.8 **M**b to *ARX* gene
		start codon	*278-280 (exon 1)*		

- Some mutations in the *RPS6KA3* gene, cause mild intellectual disability (MRX19). Most mutations (incl. truncating) cause Coffin-Lowry syndrome (CLS). CLS is characterised by (amongst others) intellectual disability, fleshy hands and prominent ears. For more information on Coffin-Lowry syndrome see https://www.ncbi.nlm.nih.gov/books/NBK1346/.
- P259 RPS6KA3: contains more probes for the *RPS6KA3* gene.

Table 2b. *ARX* gene, Xp21.3

Length (nt)	SALSA MLPA probe	ARX Exon[a]	Ligation site NM_139058.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*1915-1917 (exon 5)*		
222 «	02898-L04200	Exon 4	1652-1651 reverse	GGCTGATGAAAG-CTGGGTGTCGGA	5.9 kb
195 «	18790-L24221	Exon 2	1168-1167 reverse	GCTGCCCGCAGA-GAGGCACACGCT	2.8 kb
229 «	13669-L15822	Exon 1	115-116	AGATCGCAATAA-TATCCGTTATAA	3.6 **M**b to *IL1RAPL1* gene
		start codon	*229-231 (exon 1)*		

- Mutations in the *ARX* gene underlie a phenotypic spectrum and can cause X-linked infantile spasm syndrome (https://omim.org/entry/308350), lissencephaly (https://omim.org/entry/300215), Proud syndrome (https://omim.org/entry/300004), intellectual disability (https://omim.org/entry/300419) and Partington syndrome (https://omim.org/entry/309510).
- P189 CDKL5/ARX/FOXG1: contains more probes for the *ARX* gene.

Table 2c. *IL1RAPL1* gene, Xp21.3 – p21.2

Length (nt)	SALSA MLPA probe	IL1RAPL1 Exon[a]	Ligation site NM_014271.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*627-629 (exon 2)*		
292	02920-L02314	Exon 1	363-364	CAGCAAACAATC-GGGCACTTTGAG	201.5 kb
328	02921-L02315	Exon 2	636-637	AGATGAAAGCTC-CGATTCCACACT	493.7 kb
235	02922-L23556	Exon 3	834-835	TTGCCCAAAGTG-CTGGACTCAGTT	385.4 kb
427	02923-L23561	Exon 6	1344-1345	CTCTGACTGATA-AGCCACCCAAGC	8.7 **M**b to *TSPAN7* gene
		stop codon	*2715-2717 (exon 11)*		

- Mutations and/or deletions in the *IL1RAPL1* gene have been identified in families with X-linked non-syndromic intellectual disability (MRX21, see https://www.omim.org/entry/300143).

Table 2d. *TSPAN7* gene, Xp11.4

Length (nt)	SALSA MLPA probe	TSPAN7 Exon[a]	Ligation site NM_004615.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*6-8 (exon 1)*		
160 «	02903-L02297	Exon 1	32-33	ATGGAGACCAAA-CCTGTGATAACC	114.1 kb
268 ~	02904-L23558	Exon 5	467-468	TGTGGTGTGCAG-AACTACACCAAC	10.2 **M**b to *PQBP1* gene
		stop codon	*753-755 (exon 7)*		

- (Truncating) mutations in the *TSPAN7* gene have been identified as the cause of intellectual disability (MRX58, see https://www.omim.org/entry/300210).

Table 2e. *PQBP1* gene, Xp11.23

Length (nt)	SALSA MLPA probe	PQBP1 Exon[a]	Ligation site NM_005710.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*255-257 (exon 1)*		
309	22016-L02878	Exon 1	155-156	AGATGAGTACAT-GTTTACGGGAGG	3.8 kb
392	22856-L32371	Exon 4	567-568	AAAAGTTGGACC-GGAGCCATGACA	5.0 **M**b to *HUWE1* gene
		stop codon	*1050-1052 (exon 6)*		

- Mutations in the *PQBP1* gene have been identified as the cause of Renpenning syndrome (https://www.omim.org/entry/309500).
- P259 RPS6KA3: contains more probes for the *PQBP1* gene.

Table 2f. *HUWE1* gene, Xp11.22

Length (nt)	SALSA MLPA probe	HUWE1 Exon[a]	Ligation site NM_031407.7	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*13516-13518 (exon 84)*		
184	13919-L15456	Exon 61	8669-8670	ATCTGAGTCCAA-GGAGACCCTTGG	92.6 kb
256	13920-L23672	Exon 6	600-601	GCAGATGCTGGA-CAGACAGTGGAG	13.5 **M**b to *OPHN1* gene
		start codon	*394-396 (exon 4)*		

- Mutations in the *HUWE1* gene have been identified as the cause of Turner type of X-linked syndromic intellectual disability (MRXST, see https://omim.org/entry/309590). A nonsyndromic form of X-linked intellectual disability (MRX17 or MRX31, see https://www.omim.org/entry/300705) is caused by microduplications of chromosome Xp11.22, which includes the *HUWE1* gene.

Table 2g. *OPHN1* gene, Xq12

Length (nt)	SALSA MLPA probe	OPHN1 Exon[a]	Ligation site NM_002547.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2739-2741 (exon 24)*		
472	02915-L02309	Exon 21	2209-2210	TATCACCAGCAG-CATAGAACCCCC	133.1 kb
436	02914-L02308	Exon 12	1389-1390	AGGCCCTTTCAG-AAGCTAACAGAA	101.8 kb
409 #	02913-L23560	Exon 3	527-528	CAGACGCTGCAG-TCATTTCAGTTT	134.3 kb
371	02912-L02306	Exon 1	173-174	TGCTGCTTATCT-GGGAAGGCGATG	41.2 **M**b to *ACSL4* gene
		start codon	*333-335 (exon 2)*		

- Mutations/deletions in the *OPHN1* gene cause X-linked intellectual disability with distinctive facial appearance and cerebellar hypoplasia (https://omim.org/entry/300486).

Table 2h. *ACSL4* gene, Xq22.3

Length (nt)	SALSA MLPA probe	ACSL4 Exon[a]	Ligation site NM_022977.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2504-2506 (exon 17)*		
301	22783-L32128	Exon 17	2473-2474	AACCATTACCTC-AAAGACATTGAA	24.1 kb
148 «	02935-L02326	Exon 12	1745-1746	ATGTCTGCTTCT-GCTGCCCAATTG	65.1 kb
248 «	03512-L23557	Exon 1	38-39	GTCCCAGCGCTA-GCGGGCACGCGG	1.4 **M**b to *PAK3* gene
		start codon	*371-373 (exon 4)*		

- It has been suggested that mutations in the *ACSL4* gene might play a role in the development of intellectual disability (MRX63, see https://www.omim.org/entry/300387).

Table 2i. *PAK3* gene, Xq22.3

Length (nt)	SALSA MLPA probe	PAK3 Exon[a]	Ligation site NM_002578.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*578-580 (exon 5)*		
385	02908-L03178	Exon 5	655-656	CGGGATTCTTCA-GCACTCAACCAC	40.5 kb
418	02909-L02303	Exon 10	1252-1253	CCACCCTCTGCT-GAAAATGCCAAT	52.8 kb
400	03521-L02304	Exon 17	2014-2015	ACTAATGGAACT-CCAGAGCTCCAG	4.0 kb
481	02911-L02305	Exon 18	2185-2184 reverse	TTAATTGCTTCC-TTTGCAGCGATA	113 kb to *DCX* gene
		stop codon	*2210-2212 (exon 18)*		

- Mutations in the *PAK3* gene have been reported as being the cause of non-syndromic intellectual disability (MRX30 or MRX47, see https://www.omim.org/entry/300558).

Table 2j. *DCX* gene, Xq22.3 – q23

Length (nt)	SALSA MLPA probe	DCX Exon[a]	Ligation site NM_178152.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*1173-1175 (exon 7)*		
275	04124-L03481	Exon 4	824-825	GATGATGTGTTT-ATTGCCTGTGGT	67.9 kb
215	04123-L15828	Exon 3	716-717	GTCCTCACTGAT-ATCACAGAAGCC	9.8 kb
319	04121-L08390	Exon 2	431 nt before exon 2; NM_000555.3; 295-296 (exon 1).	CAGGCTATGGAT-TCATTTACAACT	4.7 **M**b to *AGTR2* gene
		start codon	*78-80 (exon 2)*		

- Mutations in the *DCX* gene are found to result in lissencephaly ('smooth brain'), characterised by intellectual disability and seizures. For more information on *DCX*-related disorders see https://www.ncbi.nlm.nih.gov/books/NBK1185/.
- P061 Lissencephaly: contains more probes for the *DCX* gene.

Table 2k. *AGTR2* gene, Xq23

Length (nt)	SALSA MLPA probe	AGTR2 Exon[a]	Ligation site NM_000686.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*169-171 (exon 3)*		
355	02925-L02319	Exon 1	31-32	TGAGAGAACGAG-TAAGCACAGAAT	2.1 kb
130	13917-L02320	Exon 3	773-774	TTTCCCACCTGA-GAAATATGCCCA	20.4 **M**b to *ARHGEF6* gene
		stop codon	*1258-1260 (exon 3)*		

- Mutations in the *AGTR2* gene have been reported as being the cause of non-syndromic intellectual disability (MRX88, see https://www.omim.org/entry/300852), often accompanied by seizures.

Table 2l. *ARHGEF6* gene, Xq26.3

Length (nt)	SALSA MLPA probe	ARHGEF6 Exon[a]	Ligation site NM_004840.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		stop codon	*2379-2381 (exon 22)*		
202	02902-L04460	Exon 19	2027-2028	GATGCTCAAATC-CTTAAAGTGATC	31.9 kb
172	16857-L19651	Exon 9	1002-1003	ACAAAGTAGGAG-GTTGTCTACTGA	38.3 kb
443	16856-L19650	Exon 4	417-418	GTGGACGTTCCT-CTTCTCTTAGTG	35.5 kb
343	22017-L02293	Exon 1	120-121	CTAAAAAGACCA-TCTGTGATCCGG	11.1 **M**b to *FMR1* gene
		start codon	*51-53 (exon 1)*		

- Mutations in the *ARHGEF6* gene have been reported as being the cause of non-syndromic intellectual disability (MRX46, https://www.omim.org/entry/300436).

Table 2m. *FMR1* gene, Xq27.3

Length (nt)	SALSA MLPA probe	FMR1 Exon[a]	Ligation site NM_002024.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*262-264 (exon 1)*		
166 «	02927-L03721	Exon 9	1091-1092	AAAAGCTAGAAG-CTTTCTCGAATT	12.9 kb
142	02928-L03720	Exon 16	1939-1940	ACTCCCGAACAG-ATAATCGTCCAC	556 kb to *AFF2* gene
		stop codon	*2158-2160 (exon 17)*		

- Defects in the *FMR1* gene, result in fragile X syndrome, characterised by moderate to severe intellectual disability. Expansion of a trinucleotide repeat in exon 1 of the *FMR1* gene is the most common defect of this gene. This expansion can result in silencing of the gene due to methylation of the promoter sequence. For more information on *FMR1* disorders see https://www.ncbi.nlm.nih.gov/books/NBK1384/.
- ME029 FMR1/AFF2: this methylation-specific probemix contains more probes for the *FMR1* gene and allows detection of both copy number changes, as well as the detection of promoter methylation (in full mutation male samples) of the *FMR1* and *AFF2* genes. It is not possible to directly measure the length of the trinucleotide repeat by MLPA.

Table 2n. *AFF2* gene, Xq28

Length (nt)	SALSA MLPA probe	AFF2 Exon[a]	Ligation site NM_002025.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*482-484 (exon 1)*		
154 «	03511-L04202	Exon 1	503-504	TCGACTTTTTCA-GAGACTGGGACT	161.1 kb
283	00493-L00066	Exon 3	980-981	GTCATAACCCTA-GCACTGTACTGG	175.5 kb
241	03516-L15823	Exon 5	1606-1607	CTCACTTCCATG-CATACTGCTGGA	118.4 kb
337	02932-L02323	Exon 11	2539-2540	GAACCAAGACCT-AACATCCCTTTG	31.3 kb
263	02933-L23673	Exon 20	4130-4131	CAGTGTCTCTCA-ACAACGTCTCCC	4.7 **M**b to *SLC6A8* gene
		stop codon	*4415-4417 (exon 21)*		

- The long *AFF2* gene is located at close distance (550 kb) from *FMR1* and spans almost 500 kb. Similar to *FMR1*, expansion of a trinucleotide repeat in exon 1 of the *AFF2* gene can result in inactivation of the gene. Inactivation of the *AFF2* gene has been associated with intellectual disability (FRAXE, see https://www.omim.org/entry/309548), premature ovarian failure and obsessive-compulsive disorder.
- ME029 FMR1/AFF2: this methylation-specific probemix contains more probes for the *AFF2* gene and allows detection of both copy number changes, as well as the detection of promoter methylation of the *AFF2* gene. It is not possible to directly measure the length of the trinucleotide repeat by MLPA.

Table 2o. *SLC6A8* gene, Xq28

Length (nt)	SALSA MLPA probe	SLC6A8 Exon[a]	Ligation site NM_005629.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*650-652 (exon 1)*		
208 « #	01871-L15827	Exon 3	1224-1225	AGACTGTGCCAA-TGCCAGCCTGGC	3.7 kb
188 « #	22782-L32127	Exon 13	2555-2554 reverse	GCTGAGTTGTCA-CATGACACTCTC	705 kb to *GDI1* gene
		stop codon	*2555-2557 (exon 13)*		

- Mutations in the *SLC6A8* gene are reported to cause cerebral creatine deficiency syndrome 1 characterised by intellectual disability (see https://www.omim.org/entry/300352). For more information on creatine deficiency syndromes see https://www.ncbi.nlm.nih.gov/books/NBK3794/.
- P049 SLC6A8 - ABCD1: contains more probes for the *SLC6A8* gene.

Table 2p. *GDI1* gene, Xq28

Length (nt)	SALSA MLPA probe	GDI1 Exon[a]	Ligation site NM_001493.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*102-104 (exon 1)*		
136 «	16875-L19669	Exon 1	106-107	CCTGACCATGGA-CGAGGAATACGA	3.9 kb
378 «	16874-L23559	Exon 7	876-877	TGGATGACATCA-TCATGGAGAACG	
		stop codon	*1443-1445 (exon 11)*		

- Mutations in the *GDI1* gene can cause X-linked intellectual disability (MRX41 or MRX48, see https://www.omim.org/entry/300849).

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
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.
~ More variable. This probe has been reported to be deleted/duplicated in healthy individuals (various reports).

Related SALSA MLPA probemixes

- P245 Microdeletion Syndromes-1A / P064 Microdeletion Syndromes-1B: Probes are included for different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
- P036 Subtelomeres Mix 1 / P070 Subtelomeres Mix 2B: These probemixes each contain one probe for every subtelomere.
- Several syndrome-specific probemixes useful for confirmation of findings with this P106 X-linked ID probemix are available; see Table 2.

References

- Ropers HH, Hamel BC. (2005) X-linked mental retardation. *Nat Rev Genet*. 6(1):46-57. doi: 10.1038/nrg1501. PMID: 15630421.
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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

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- Flynn, M. et al. (2011). Whole gene duplication of the PQBP1 gene in syndrome resembling Renpenning. *Am J Med Genet. A.* 155A:141-4.
- Madrigal, I. et al. (2007). MLPA as first screening method for the detection of microduplications and microdeletions in patients with X-linked mental retardation. *Genet Med*. 9:117-22.
- Tejada, M. et al. (2011). A child with mild X-linked intellectual disability and a microduplication at Xp22.12 including RPS6KA3. *Pediatrics.* 128:e1029
- Utine, G.E. et al. (2012). Searching for copy number changes in nonsyndromic X-linked intellectual disability. *Mol Syndromol.* 2:64-71.

P106 Product history	
Version	*Modification*
D1	Two probes have been replaced, three probes have been changed in length not in sequence detected.
C1	One *ARX* probe has been replaced and the Y-chromosome fragment on 118 nt has been elongated to 121 nt.
B2	One *RPS6KA3* probe has been removed and the Y-chromosome fragment on 118 nt and the control fragments (QDX2) have been replaced.
B1	Two probes for the *HUWE1* gene and one extra *AGTR2* probe have been included. In addition two *ARX* probes and one *SLC6A8* probe have been replaced. Finally, extra control fragments at 88-96-100 and 105 nt have been included.
A1	First release.

Implemented changes in the product description

Version D1-01 — 21 October 2020 (02P)
- Product renamed from "MRX" to "X-linked ID".
- "Intellectual disability" has replaced the term "mental retardation" throughout this document.
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Various minor textual or layout changes.
- Ligation sites of the probes targeting all genes except for *PQBP1* updated according to new version of the NM_ reference sequence.
- Warning added to Table 1 and 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).

Version 18 – 30 September 2016 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Several small textual changes throughout the document.

Version 17 – 29 February 2016 (55)
- DCX and SLC6A8 exon numbering adjusted in Table 2j and 2o, respectively.

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