Product Description
SALSA® MLPA® Probemix P095-A4 Aneuploidy

To be used with the MLPA General Protocol.

Version A4
For complete product history see page 9.

Catalogue numbers:
- **P095-025R:** SALSA MLPA Probemix P095 Aneuploidy, 25 reactions.
- **P095-050R:** SALSA MLPA Probemix P095 Aneuploidy, 50 reactions.
- **P095-100R:** SALSA MLPA Probemix P095 Aneuploidy, 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 P095 Aneuploidy is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications of eight DNA sequences on each of four human chromosomes (13, 18, 21, X) and four sequences on chromosome Y as a means to detect an aneuploidy for one of these chromosomes in prenatal and postnatal DNA samples. For postnatal samples, genomic DNA isolated from human peripheral whole blood specimens or buccal swabs can be used. For prenatal samples, genomic DNA isolated from (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 can be used. P095 Aneuploidy is intended to confirm a potential cause for and clinical diagnosis of Patau, Edwards and Down syndromes (trisomy 13, 18 and 21, respectively) and Turner, Triple X, Klinefelter, 47,XYY syndromes (X/Y chromosome aneuploidies). This probemix cannot distinguish between normal females (46,XX) and triploid females (69,XXX).

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, 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 or 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.

Clinical background
Germline aneuploidy of a complete chromosome is the main cause of spontaneous pregnancy loss. The most common cases of an abnormal chromosome copy number at birth concern the presence of an extra chromosome 13, 18 or 21 (leading to Patau, Edwards and Down syndromes, respectively), each resulting in

intellectual disability and various syndromic birth defects. The presence of an extra or missing sex chromosome e.g. X0, XXX, XXY, XYY, results generally in less severe consequences (Turner, Triple X, Klinefelter, and XYY syndromes respectively). Full chromosome gain or loss account for the majority of cases, while a minority results from partial chromosome duplications or mosaicism.

More information can be found on Decipher (http://decipher.sanger.ac.uk/) and in the references listed at the end of this product description. For more information about a specific syndrome, the OMIM and Genetics Home Reference databases can be consulted:
https://medlineplus.gov/genetics/condition/trisomy-13/ Patau syndrome (Trisomy 13)
https://medlineplus.gov/genetics/condition/trisomy-18/ Edwards syndrome (Trisomy 18)
https://medlineplus.gov/genetics/condition/down-syndrome/ Down syndrome (Trisomy 21)
https://medlineplus.gov/genetics/condition/klinefelter-syndrome/ Klinefelter syndrome (47,XXY)

Probemix content
This SALSA MLPA probemix P095-A4 Aneuploidy contains 36 MLPA probes with amplification products between 136 and 454 nucleotides (nt). This includes eight probes for each chromosome 13, 18, 21, and X; and four probes for the Y chromosome. More information is present in Table 2 of this product description. Complete probe sequences are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity Fragments (Q-fragments), two DNA Denaturation Fragments (D-fragments), one benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com

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

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

MLPA technique validation
Internal validation of the MLPA technique using 16 male DNA samples (20 ng each) 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, or foetal blood, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated **male** individuals without developmental delay

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. Sample ID numbers NA02948, NA02422, AG05024 and NA04375 from the Coriell Institute have been tested with P095-A4 probemix at MRC Holland and can be used as a positive control samples to detect trisomy 13, 18, 21 and Klinefelter variant XXYY, respectively. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID	Source	Probes affected	Expected alteration
NA02948	Coriell Institute	*All probes targeting chr. 13*	Trisomy chr. 13
NA02422	Coriell Institute	*All probes targeting chr. 18*	Trisomy chr. 18
AG05024	Coriell Institute	*All probes targeting chr. 21*	Trisomy chr. 21
NA04375	Coriell Institute	*All probes targeting chr. X and chr. Y*	Klinefelter variant 48,XXYY

Performance characteristics

Patau, Edwards and Down syndromes are caused and defined by the presence of trisomy 13, 18 and 21, respectively. The diagnosis for these three trisomies, as well as the diagnosis of Turner, Triple X, Klinefelter and other sex chromosome syndromes, is dependent on the presence of the corresponding aneuploidy. There are no other (non)genetic causes for these syndromes, except for some rare cases of partial aneuploidy and mosaicism.

Several studies have reported a diagnostic and analytical sensitivity and specificity >99% for the detection of chromosomes 13, 18, 21, X and Y aneuploidies using MLPA probemix P095 Aneuploidy, with a 100% accuracy for the detection of full trisomies 13, 18 and 21 (e.g. Boormans et al. 2010, Gerdes et al. 2008, Kooper et al. 2008, and Van Opstal et al. 2009).

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

In probemix P095, all autosomal probes detecting loci on chromosomes 13, 18, and 21 are set as reference probes. In full trisomy cases, the values for all eight reference probes detecting the same chromosome will therefore be increased. During intra-sample normalisation, each probe signal is compared with each of the 24 reference probe signals. The Coffalyser.Net software neutralizes the effect of an increased value for 8 out of 24 reference probes in trisomy samples by calculating the median of the 24 normalised values of each probe. The expected final ratio (FR)-values for various genotypes as determined by Coffalyser.Net software can be found in Table 1 below.

Table 1. P095 final ratio expected in aneuploidy and normal cases when using <u>all</u> chromosome 13, 18, and 21 probes as reference probes and using 46,XY as reference samples.

Sample type		chr. 13 probes	chr. 18 probes	chr. 21 probes	chr. X probes	chr. Y probes
46,XY	Normal male	1	1	1	1	1
46,XX *	Normal female	1	1	1	2	0
47,XY,+13	Patau syndrome	1.5	1	1	1	1
47,XY,+18	Edwards syndrome	1	1.5	1	1	1
47,XY,+21	Down syndrome	1	1	1.5	1	1
45,X0	Turner syndrome	1	1	1	1	0
47,XXX	Triple X syndrome	1	1	1	3	0
47,XXY	Klinefelter syndrome	1	1	1	2	1
47,XYY	XYY syndrome	1	1	1	1	2
69,XXX *	Triploid female	1	1	1	2	0
69,XXY	Triploid male	1	1	1	1.33	0.67
69,XYY	Triploid male	1	1	1	0.67	1.33

* MLPA cannot distinguish between 69,XXX and 46,XX samples.

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.

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 detecting normal copy number. When these criteria are fulfilled, the following criteria can be used to interpret MLPA results:

<u>For copy number changes of a complete chromosome (majority of cases):</u>
In theory, all probes targeting a certain chromosome should give approximately the same FR-value, also in cases of aneuploidy. In practice, however, certain variables such as SNPs, sample impurities etc., can affect this. Based on Van Opstal et al. (2009), we recommend the following rules for determining copy number status:

1. A trisomy for a particular chromosome should be considered when the following two criteria are met:
   I. At least 4 out of 8 probe ratios for a certain chromosome are ≥ 1.30 AND
   II. At least 6 out of 8 probes ratios for that chromosome are ≥ 1.20
2. Monosomy X should be considered if the probe ratios for X chromosome probes are within the range of those of normal males and Y signals are absent.
3. 47,XXY should be considered if probe ratios for Y chromosome probes are within the range for those of normal males and X chromosome probes are ~2 times those of normal males.
4. 47,XYY should be considered if probe ratios for Y chromosome probes are ~2 times those of normal males and X chromosome probes are within the range of those of normal males.
5. 47,XXX should be considered if the relative probe signals for X chromosome probes are ~2.5-3 times those of normal males and Y signals are absent.

For partial chromosome gain or mosaic cases (minority of cases):

1. In case a partial chromosome gain has occurred, a duplication of a specific region will result in a FR > 1.30 of two or more of the corresponding probes. Additional studies should be used to confirm and interpret the result.
2. We recommend a follow-up study for samples in which several probes for a certain chromosome are marked as having a statistically abnormal probe value by Coffalyser.Net analysis. The use of a 1.30 cut-off value to distinguish a normal from a trisomy result does not allow detection of mosaic samples. As an example, all chromosome 21 specific probes may have a ratio between 1.10 and 1.20 in a mosaic sample with 30% trisomy 21 cells. Coffalyser.Net analysis may identify such mosaic samples when the experiment was performed well.

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

P095 specific notes:

- This probemix is validated to be used with 20-250 ng DNA input.
- DNA from whole genome amplification reactions (WGA) is not suitable for MLPA due to amplification bias.
- MLPA cannot distinguish between normal females (46,XX) and triploid females (69,XXX).
- As the P095 probe sequences have been selected based on their location on a specific chromosome, not on being located in a specific gene, we recommend to disregard copy number alterations detected by a single probe. Copy number changes detected by only a single probe may be a false positive result due to e.g. a mutation or polymorphism that prevents ligation of the MLPA probe or that destabilizes the binding of a probe oligonucleotide to the sample DNA.

Limitations of the procedure

- For use on (un)cultured amniocytes and (un)cultured chorionic villi, contamination of the sample with maternal DNA may lead to wrong conclusions. Contamination of foetal DNA samples with maternal DNA can be detected by QF-PCR, but not by MLPA.
- MLPA cannot discriminate maternal and foetal DNA in one sample, therefore, it is not possible to use maternal blood samples for prenatal diagnosis.
- For use on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and foetus 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).
- 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 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 more than one consecutive probe should be confirmed by another independent technique such as karyotyping, FISH, array CGH or low coverage whole genome DNA sequencing, whenever possible.

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 2. SALSA MLPA Probemix P095-A4 Aneuploidy

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Location (hg18) in kb	Partial sequence[a] (24nt adjacent to ligation site)
64-105	Control fragments – see table in probemix content section for more information			
136 «	00815-L00333	21q22.13	21-037,044	GATTCTTGAAAG-GTGTAGGTTTGA
142	02127-L01638	18q21.2	18-046,827	GCATTGTGCATA-GTTTGATGTGCC
148	00798-L00316	13q32.1	13-094,526	ATCCGGGCATAC-AAAGCAGAAGAG
154 «	00652-L00637	Xq12	X-066,682	CATGCAACTCCT-TCAGCAACAGCA
160	02153-L00596	Yp11.31	Y-002,715	GCACTGAAAGCT-GTAACTCTAAGT
166	00813-L00636	21q21.1	21-021,726	AGAGCTGTCGCA-GACCACGGCCAA
172	00808-L00326	18q21.32	18-055,721	CTCAGGAGATTT-GGAGACAAACTG
178	00799-L00317	13q13.3	13-035,914	GTATTCATTAAG-TGAAATTGTGCC
184	02155-L01607	Xq22.3	X-108,774	TAAGCCCAGAGC-CATGGACCCCTG
193	02152-L00592	Yp11.31	Y-002,715	CAACAGGTTGTA-CAGGGATGACTG
202	02115-L01605	21q21.1	21-016,173	CTGTGAGCGATT-TGCCCGAATCAT
211	02845-L02275	18q11.2	18-021,892	CATGCCTTCAGA-TGGAATGGTAGG
220	00582-L00147	13q14.2	13-047,952	GTGGACACTGTG-TACACCTCTGGA
229 «	02898-L02369	Xp21.3	X-024,935	GGCTGATGAAAG-CTGGGTGTCGGA
240	01071-L00464	Yq11.21	Y-014,101	CTTCGGTAGCTT-AAGTCTTTGCCT
247	00816-L00334	21q11.2	21-014,675	GATATGAAAGCG-TAGAGCTGGCAG
255	00807-L00325	18q23	18-075,328	ACCAGCCCCGTT-CACGTCAGTTTC
265	02125-L01636	13q21.33	13-071,154	AGAGGACTCAAA-GTGTCACCTCCC
274	02904-L02370	Xp11.4	X-038,420	TGTGGTGTGCAG-AACTACACCAAC
283	04189-L03982	Yp11.31	Y-002,889	TCATAGAGGAGG-ATGTTCAGTGCT
291	02116-L01604	21q22.11	21-031,961	AATAGACACATC-GGCCACACCATC
301	00811-L00329	18p11.32	18-000,663	CTTCGAAAAGTT-GAGAAAATTGAT
310	00871-L00461	13q34	13-110,756	CTGGTTGTGTGG-ACTCCTTAATGC
319 «	00819-L28681	Xq28	X-152,791	AACAGCAACTTT-GCTCAGAGGTTC
337	00812-L00330	21q21.3	21-026,192	AGATGGATGCAG-AATTCCGACATG
346	00810-L00328	18q21.33	18-059,714	TGGGTTCATGCA-GCAGATCCAGAA
355	00452-L00360	13q13.1	13-031,829	GCGTCTTTCCAC-AGCCAGGCAGTC
364	02906-L02300	Xp22.12	X-020,137	AAGGAAGGACAT-GAAAAGGCAGAT
382	02834-L02265	21q22.3	21-042,660	CCACCATGGAGA-ACAAGGTGATCT
391	02846-L02276	18q11.2	18-021,873	ATGGGAATGATG-GGTCAAGTTAAC
400	00801-L00639	13q14.3	13-049,577	GAAGAACAGAAC-CTTCAGGAATTG
409	00820-L28679	Xq25	X-129,093	TATTGGTCTTGT-GGACAGTAGTTT
427	00817-L00335	21q22.11	21-031,425	TGGTCCTTGTGT-ATAAAGATGGTT
436	02847-L02277	18p11.21	18-013,875	AGTAACCCCTAC-TGCGCCTGCTAC
445 «	00802-L00320	13q34	13-110,170	GCTACGAGCGCT-TCAGTCGCGAGA
454	01388-L28680	Xp21.2	X-031,108	GCTTTTTTTCTG-GTCGAGTTGCAA

[a] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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

Related SALSA MLPA probemixes

P036 Subtelomeres Mix 1 and 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 P070 and P036 probes differ from each other. We strongly recommend using both P036 and P070 on each sample.
P106 X-linked ID	Probes for several genes associated with X-linked intellectual disability.
P181 Centromeres Mix 1	Contains one probe for each of the 41 different centromeric regions and an extra probe near the centromeric region of each of the five acrocentric chromosomes, resulting in two probes for each chromosome.
P182 Centromeres Mix 2	Similar to P181 centromeres, but all probes are different from the P181 probes.
P245 Microdeletion Syndromes-1A	Probes for 23 different microdeletion syndromes; can be used for screening of microdeletion syndromes.
P064 Microdeletion Syndromes-1B	Probes for 15 different microdeletion syndromes; can be used for screening of microdeletion syndromes.
P297 Microdeletion Syndromes-2	Probes for 11 different microdeletion syndromes; can be used for screening of microdeletion syndromes.

References

- Boormans EM et al. (2010). Comparison of multiplex ligation-dependent probe amplification and karyotyping in prenatal diagnosis. *Obstet Gynecol.* 115:297-303.
- Gerdes T et al. (2008). Multiplex ligation-dependent probe amplification (MLPA) in prenatal diagnosis-experience of a large series of rapid testing for aneuploidy of chromosomes 13, 18, 21, X and Y. *Prenat Diagn.* 28:1119-1125.
- Kooper AJ et al. (2008). Multiplex ligation-dependent probe amplification (MLPA) as a stand-alone test for rapid aneuploidy detection in amniotic fluid cells. *Prenat Diagn.* 28:1004-1010.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Van den Berg C et al. (2006). (Potential) false-negative diagnoses in chorionic villi and a review of the literature. *Prenat Diagn*. 26:401-408.
- Van Opstal D et al. (2009). Rapid aneuploidy detection with multiplex ligation-dependent probe amplification: a prospective study of 4000 amniotic fluid samples. *Eur J Hum Genet.* 17:112-121.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P095 Aneuploidy

- Bocian E et al. (2011). Usefulness of MLPA technique for rapid prenatal detection of aneuploidy. Results of 409 diagnostic studies. *Ginekol Pol.* 82:680-4.
- Boormans EM et al. (2012). Economic evaluation of multiplex ligation-dependent probe amplification and karyotyping in prenatal diagnosis: a cost-minimization analysis. *Arch Gynecol Obstet.* 285:67-75.
- Carvalho B et al. (2010). Aneuploidies detection in miscarriages and fetal deaths using multiplex ligation-dependent probe amplification: an alternative for speeding up results? *Eur J Obstet Gynecol Reprod Biol.* 153:151-5.
- Chen CP et al. (2011). Rapid aneuploidy diagnosis by multiplex ligation-dependent probe amplification and array comparative genomic hybridization in pregnancy with major congenital malformations. *Taiwan J Obstet Gynecol* 50:85-94.
- Chitty LS et al. (2012). Multiplex ligation-dependent probe amplification (MLPA): a reliable alternative for fetal chromosome analysis? *J Matern Fetal Neonatal Med.* 25:1383-6.
- Diego-Alvarez D et al. (2006). Double trisomy in spontaneous miscarriages: cytogenetic and molecular approach. *Hum Reprod.* 21:958-66.
- Faas BH et al. (2011). Rapid methods for targeted prenatal diagnosis of common chromosome aneuploidies. *Semin Fetal Neonatal Med.* 16:81-7.
- Fauret AL et al. (2009). Molecular biology usefulness for rapid diagnosis of Down's syndrome and common aneuploidies. *Gynecol Obstet Ferti.* 37:611-9.
- Gerdes T et al. (2005a). Automatic analysis of multiplex ligation-dependent probe amplification products (exemplified by a commercial kit for prenatal aneuploidy detection). *Electrophoresis* 26:4327-32.
- Gerdes T et al. (2005b). Computer-assisted prenatal aneuploidy screening for chromosome 13, 18, 21, X and Y based on multiplex ligation-dependent probe amplification (MLPA). *Eur J Hum Genet.* 13:171-5.

- Grandone A et al. (2016). Multiplex Ligation-Dependent Probe Amplification Accurately Detects Turner Syndrome in Girls with Short Stature. *Hormone research in paediatrics.* 86:330-336.
- Hamidah NH et al. (2014). Prenatal diagnosis of aneuploidies in amniotic fluid by multiple ligation-dependent probe amplification (MLPA) analysis. *Malays J Pathol* 36:163-8.
- Hochstenbach R et al. (2005). Rapid detection of chromosomal aneuploidies in uncultured amniocytes by multiplex ligation-dependent probe amplification (MLPA). *Prenat Diagn.* 25:1032-9.
- Jóźwiak A et al. (2013). Effectiveness of multiplex ligation dependent probe amplification (MLPA) in prenatal diagnosis of common aneuploidies. *Ginekol Pol.* 84:682-90.
- Kooper AJ et al. (2009). Detection of chromosome aneuploidies in chorionic villus samples by multiplex ligation-dependent probe amplification. *J Mol Diagn.* 11:17-24.
- Luo SQ et al. (2011). Application of multiplex ligation-dependent probe amplification to diagnosis and prenatal diagnosis of common aneuploidies. *Zhonghua Yi Xue Chuan Xue Za Zhi.* 28:212-6.
- Ma DY et al. (2011). Application of multiplex ligation-dependent probe amplification for rapid detection of aneuploidies in prenatal diagnosis. *Zhonghua Yi Xue Chuan Xue Za Zhi.* 28:543-7.
- Massalska D et al. (2013). Multiplex ligation-dependent probe amplification (MLPA)--new possibilities of prenatal diagnosis. *Ginekol Pol.* 84:461-4.
- Ramsden SC et al. (2007). External quality assessment of rapid prenatal detection of numerical chromosomal aberrations using molecular genetic techniques: 3 years experience. *Prenat Diagn.* 27:404-8.
- Schouten JP and Galjaard RJ (2008). MLPA for prenatal diagnosis of commonly occurring aneuploidies. *Methods Mol Biol.* 444:111-22.
- Slater HR et al. (2003). Rapid, high throughput prenatal detection of aneuploidy using a novel quantitative method (MLPA). *J Med Genet.* 40:907-12.
- Srebniak MI et al. (2011). Prenatally diagnosed submicroscopic familial aberrations at 18p11.32 without phenotypic effect. *Mol Cytogenet.* 4:27.
- Tang SH et al. (2011). Rapid and high-throughput multiplex ligation-dependent probe amplification for diagnosis of chromosome aneuploidy. *Zhonghua Yi Xue Chuan Xue Za Zhi.* 28:199-203.
- Yurdakul H et al. (2010). Performance of MLPA as a screening method for aneuploidy in uncultured amniocytes. *J Turk Ger Gynecol Assoc.* 11:199-203.
- Willis AS et al. (2012). Multiplex ligation-dependent probe amplification (MLPA) and prenatal diagnosis. *Prenat Diagn*. 32:315-20.
- Zhang J et al. (2014). Application of multiplex ligation-dependent probe amplification for rapid detection of aneuploidies and structural chromosomal abnormalities in prenatal diagnosis. *Zhonghua Yi Xue Chuan Xue Za Zhi*. 31:11-5.

P095 product history	
Version	*Modification*
A4	This probemix is now suitable for use with a DNA input amount between 20-250 ng. In three probes the first nucleotide of the LPO was adjusted. No change in length or in the sequence detected by the probes.
A3	DNA denaturation control fragments at 88 and 96 nts replaced (QDX2).
A2	Four control fragments added at 88, 96, 100, and 105 nts (QDX1).
A1	First release P095. Contains 18 probes that were present in P001 and 18 new probes.
A	First release (previously named P001).

Implemented changes in the product description

Version A4-04 – 22 April 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended purpose updated.
- Positive DNA sample information updated.
- Clinical background, performance characteristics, interpretation of results, limitations of the procedure, and selected publications sections were updated.
- Warning added to Table 2 for probe 319 nt which is located in or near a GC-rich region.
- Warning removed in Table 2 about the 184 nt and 274 nt probe being variable.
- The chromosomal position of 240 nt and 400 nt probes in Table 2 were updated.

- Chromosomal band adjusted to hg18 for 184 nt and 409 nt probes.
- Related SALSA MLPA probemixes section was updated. MLPA probemixes P297 was added and P365, P249, P291 and P320 were removed.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version A4-03 – 17 June 2020 (04)
- Colombia, Israel and Morocco added as countries with IVD status.

Version A4-02 – 14 March 2018 (04)
- Product description adapted to a new template.
- Updated limitations of the procedure chapter.
- Interpretation of results for trisomy detection updated.
- Salt sensitive probes updated in Table 2.
- References to the related SALSA MLPA probemixes P069 Subtelomeres and P358 Telomeres removed, because these probemixes will be discontinued (in Q2 2018).

Version A4-01 – 21 September 2016 (03)
- Product description restructured and adapted to a new template.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

IVD	EUROPE* CE COLOMBIA ISRAEL MOROCCO
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.