Product Description
SALSA® MLPA® Probemix P015-F2 MECP2

To be used with the MLPA General Protocol.

Version F2
For complete product history see page 13.

Catalogue numbers:
- **P015-025R:** SALSA MLPA Probemix P015 MECP2, 25 reactions.
- **P015-050R:** SALSA MLPA Probemix P015 MECP2, 50 reactions.
- **P015-100R:** SALSA MLPA Probemix P015 MECP2, 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 P015 MECP2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the human *MECP2* gene, in order to confirm a potential cause for and clinical diagnosis of classic and atypical Rett syndrome, and *MECP2* duplication syndrome. It can also be used for the detection of deletions or duplications in the human *CDKL5*, *ARX* and *NTNG1* genes, in order to confirm a potential cause for and clinical diagnosis of CDKL5 deficiency disorder, early infantile epileptic encephalopathy 1 (EIEE1) and atypical Rett syndrome, respectively. This assay is additionally intended for molecular genetic testing of at-risk family members, and is for use with genomic DNA isolated from human peripheral whole blood specimens.

Copy number variations (CNVs) detected with P015 MECP2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *MECP2*, *CDKL5*, *ARX* and *NTNG1* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Not all exons of the *CDKL5*, *ARX* and *NTNG1* genes are covered. The SALSA MLPA Probemix P189 CDKL5/ARX/FOXG1 is available for the detection of deletions or duplications in each exon of *CDKL5, ARX* and *NTNG1*.

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, pre-implantation or prenatal 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 use (IVD) 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

Rett syndrome (RTT) is a neurodevelopmental disorder affecting approximately 1:10,000 live female births. Classic RTT is characterized by a period of normal development during the first 6–18 months of life, followed by loss of already gained skills, such as speech and purposeful hand movement. Additional main features are acquired microcephaly, stereotypic hand movements, impaired locomotion and communication dysfunction (Hagberg et al. 1983). Patients lacking one or more of the major features of RTT are identified as atypical RTT cases, which are traditionally subdivided into three distinct clinical subgroups: congenital, early-onset seizure, and preserved speech (Hagberg et al. 2002; Hagberg and Skjeldal 1994; Neul et al. 2010; Pini et al. 2016). The early onset seizure and congenital variants of RTT are nowadays considered distinct clinical entities: CDKL5 deficiency disorder and *FOXG1* syndrome, respectively (Fehr et al. 2013).

RTT is an X-linked dominantly inherited disorder that, in most cases, is caused by mutations of the *MECP2* gene encoding methyl-CpG-binding protein 2 (https://www.ncbi.nlm.nih.gov/books/NBK1497/; Amir et al. 1999). Mutations in *MECP2* account for 95-97% of the classic RTT cases (Neul et al. 2008; Neul et al. 2010). Approximately 3–5% of individuals who strictly meet clinical criteria for RTT do not have an identified mutation in *MECP2*, indicating that a mutation in *MECP2* is not required to make the diagnosis of classic RTT. In contrast to classic RTT, mutations in *MECP2* have been identified in only 50-70% of atypical RTT cases (Percy et al. 2007). Most cases of RTT are the result of *de novo* mutations. Approximately 5-10% of the *MECP2* mutations are large deletions/duplications (Archer et al. 2006; Hardwick et al. 2007; Pan et al. 2006; Philippe et al. 2006; Zahorakova et al. 2007). Involvement of other genes in atypical RTT has been reported. One report described a patient with atypical RTT who presented with early onset of epileptic seizures (not infantile spasms) and a *de novo* translocation that disrupted the *NTNG1* gene on chromosome 1 (Borg et al. 2005). This balanced translocation will not be detected by MLPA as the *NTNG1* copy number is not altered. Deletions and duplications of *NTNG1* have not been described so far.

CDKL5 deficiency disorder (previously classified as early onset seizure variant of RTT; also known as early infantile epileptic encephalopathy 2) is a condition characterized by a broad range of clinical symptoms and severity. The primary symptoms include early-onset epilepsy (starting within the first three months of life), generalized hypotonia, psychomotor development disorders, intellectual disability, and cortical vision disorders. CDKL5 deficiency disorder is an X-linked dominantly inherited disorder that is caused by mutations in the *CDKL5* gene (Kalscheuer et al. 2003; Scala et al. 2005; Weaving et al. 2004). The prevalence among women is four times higher than in men (Jakimiec et al. 2020), but the course of the disease is usually more severe in male patients. Most cases of CDKL5 deficiency disorder are the result of *de novo* mutations. It is estimated that ~6.5–10% of the *CDKL5* mutations are large deletions or duplications (RettBASE; RettSyndrome.org Variation Database). Mosaicism has been reported for *CDKL5* mutations with an overall frequency of 8.8% (Stosser et al. 2018). Large mosaic deletions have also been described (Bartnik et al. 2011; Boutry-Kryza et al. 2014; Mei et al. 2014), but the occurrence rate for mosaic copy number changes has not been determined.

While loss-of-function mutations in *MECP2* result in RTT, gain-of-function mutations are associated with *MECP2* duplication syndrome, which occurs almost exclusively in males. *MECP2* duplication syndrome and RTT share overlapping clinical phenotypes including intellectual disability, speech and motor delay, seizures, hypotonia, and progressive spasticity (https://www.ncbi.nlm.nih.gov/books/NBK1284/).

Early infantile epileptic encephalopathy (EIEE; also known as developmental and epileptic encephalopathy) is a neurological disorder characterized by seizures. The disorder affects male and female newborns, usually within the first three months of life (most often within the first 10 days) in the form of epileptic seizures. Most infants with the disorder show underdevelopment of part or all of the cerebral hemispheres or structural anomalies. EIEE can be caused by mutations in more than 100 different genes. EIEE1 is an X-linked recessive disease that is caused by mutations in the *ARX* gene. Males with *ARX* mutations are often more severely affected than females, but female mutation carriers may also be affected (Kato et al. 2004; Wallerstein et al. 2008). Approximately 3% of identified *ARX* mutations are large deletions and duplications (Shoubridge et al. 2010).

Since there are multiple genes involved in the above-described syndromes and since these genes are covered by two different probemixes, i.e. SALSA MLPA Probemix P015 MECP2 and SALSA MLPA Probemix P189 CDKL5/ARX//FOXG1, the table below provides an overview of conditions and genes covered by SALSA MLPA Probemix P015-F2 MECP2 and SALSA MLPA Probemix P189-C2 CDKL5/ARX/FOXG1.

Condition	Genes	Probemix and coverage	Remarks
Classic Rett syndrome	*MECP2* (4 exons)	**P015-F2**: Each exon	-
***MECP2* duplication syndrome**	*MECP2* (4 exons)	**P015-F2**: Each exon	-
Atypical Rett syndrome	*MECP2* (4 exons)	**P015-F2**: Each exon	-
	NTNG1 (6 exons)	P189-C2: Each exon **P015-F2**: Exons 2, 3, 5, 6	Exon 3, 5 and 6 probes in P015 have the same ligation site as probes in P189.
CDKL5 deficiency disorder	*CDKL5* (21 exons)	P189-C2: Each exon **P015-F2**: Exons 3, 6, 9, 10	Probes in P015 have the same ligation sites as probes in P189.
Early infantile epileptic encephalopathy 1	*ARX* (5 exons)	P189-C2: Each exon **P015-F2**: Exons 1, 5	Probes in P015 have the same ligation sites as probes in P189.
***FOXG1* syndrome**	*FOXG1* (1 exon)	P189-C2: Exon 1 and upstream region	-

Gene structure

The *MECP2* gene spans ~76 kilobases (kb) on chromosome Xq28 (reverse strand) and contains 4 exons. The *MECP2* LRG_764 is available at www.lrg-sequence.org and is identical to GenBank NG_007107.3.

The *CDKL5* gene spans ~228 kb on chromosome Xp22.13 and contains 21 exons.

The *ARX* gene spans ~12 kb on chromosome Xp21.3 and contains 5 exons.

The *NTNG1* gene spans ~342 kb on chromosome 1p13.3 and contains 6 exons.

Transcript variants

For *MECP2*, multiple variants have been described. Transcript variant 1, also known as MECP2A, is a reference standard in the NCBI RefSeq project (NM_004992.4; 10467 nt; coding sequence 213-1673, https://www.ncbi.nlm.nih.gov/gene/4204). This transcript variant includes exon 2 and encodes isoform 1. Transcript variant 2 (MECP2B; NM_001110792.2) and transcript variants 3-7 are shorter variants compared to transcript variant 1. Transcript variant 2 lacks exon 2 and encodes isoform 2. Transcript variants 3-7 have different exon compositions but all encode the same isoform 3.

For *CDKL5,* multiple variants have been described. Transcript variant 1 (NM_003159.3; 3428 nt; coding sequence 251-3343, https://www.ncbi.nlm.nih.gov/gene/6792) is 1 nt longer than transcript variant 2 (NM_001037343.2) and shorter than transcript variant 3 (NM_001323289.2).

For *ARX,* one transcript variant has been described encoding the full length protein (NM_139058.3; 2893 nt; coding sequence 229-1917, https://www.ncbi.nlm.nih.gov/gene/170302). This sequence is a reference standard in the NCBI RefSeq project.

For *NTNG1,* multiple variants have been described. Transcript variant 3 (NM_014917.4; 6037 nt; coding sequence 638-1954, https://www.ncbi.nlm.nih.gov/gene/22854) is a reference standard in the NCBI RefSeq project. It contains a longer 5' UTR and lacks two exons in the 3' coding region, but maintains the reading frame, as compared to variant 1 (NM_001113226.3). The encoded isoform (G1c) is shorter than isoform G1a.

Exon numbering
The exon numbering used in this P015-F2 MECP2 product description is the exon numbering from LRG_764 for *MECP2*, from NG_008475.1 for *CDKL5*, from NG_008281.1 for *ARX*, and from NG_042821.1 for *NTNG1*. The *MECP2* exon numbering has changed; the exon numbering used in previous versions of this product description can be found in between brackets in Table 3a. From product description version F2-04 onwards, we have adopted the LRG sequence exon numbering. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG or NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P015-F2 MECP2 contains 46 MLPA probes with amplification products between 130 and 467 nucleotides (nt). This includes 17 probes for the *MECP2* gene, covering every exon. Furthermore, nine probes are present for genes in close proximity to *MECP2*. One of these probes detects the *VAMP7* gene that is located within the pseudo-autosomal region 2 (PAR2). The P015-F2 MECP2 probemix also contains four *CDKL5* probes, two *ARX* probes, and four *NTNG1* probes. More probes for the *CDKL5*, *ARX* and *NTNG1* genes are present in SALSA MLPA Probemix P189 CDKL5/ARX/FOXG1. In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

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

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

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

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals 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 from human peripheral whole blood specimens, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

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

history of developmental delay or epilepsy. Importantly, all reference samples need to be of the same sex (all male, or all female) for correct data analysis. In addition, it is recommended to use a set of reference samples of the same sex as patient samples, for ease of interpretation.More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers from the Coriell Institute as described in the table below have been tested with this P015-F2 probemix at MRC Holland and can be used as a positive control samples. The quality of cell lines can change; therefore samples should be validated before use.

Sample ID number	Gender	Source	Expected copy number variation
NA23599	female	Coriell Institute	Heterozygous *MECP2* exon 3-4 deletion
NA23635	female	Coriell Institute	Heterozygous *MECP2* exon 3 and partial exon 4 deletion
NA23648	female	Coriell Institute	Heterozygous *MECP2* partial exon 4 deletion that extends into *IRAK1*
NA23654	female	Coriell Institute	Heterozygous *MECP2* exon 3 and partial exon 4 deletion
NA23733	female	Coriell Institute	Heterozygous duplication of *FLNA, MECP2, IRAK1* and *L1CAM*
NA23734	male	Coriell Institute	Duplication of *FLNA, MECP2, IRAK1* and *L1CAM*
NA23676	female	Coriell Institute	Heterozygous duplication of *MECP2* and *IRAK1*

Performance characteristics

Deletions and duplications in *MECP2* explain 5-10% of the RTT cases (Archer et al. 2006; Hardwick et al. 2007; Pan et al. 2006; Philippe et al. 2006; Zahorakova et al. 2007), and duplications of *MECP2* cause 100% of the *MECP2* duplication syndrome cases (https://www.ncbi.nlm.nih.gov/books/NBK1284/). Approximately 6.5–10% of the patients with CDKL5 deficiency disorder have large deletions or duplications in the *CDKL5* gene (RettBASE; RettSyndrome.org Variation Database), and approximately 3% of the *ARX* mutations identified are large deletions or duplications (Shoubridge et al. 2010). No deletions or duplications in the *NTNG1* gene have been described so far. However, a translocation disrupting the *NTNG1* gene has been described as the genetic cause in a patient with atypical RTT (Borg et al. 2005). The analytical sensitivity and specificity for the detection of deletions or duplications in the *MECP2*, *CDKL5*, *ARX* and *NTNG1* genes is very high and can be considered >99% (based on a 2003-2021 literature review).

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

Data analysis

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

Interpretation of results

The expected results for the probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). The same results can be expected for the X-chromosome-specific probes in female samples. For the X-chromosome-specific probes in male samples, expected copy numbers are 1 (normal), 0 (deletion) or 2 (duplication).

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

Copy number status		**Final ratio (FR)**
(Pseudo-)autosomal sequences and X chromosome sequences in females	**X chromosome sequences in males**	
Normal	Normal	0.80 < FR < 1.20
Homozygous deletion	Deletion	FR = 0
Heterozygous deletion		0.40 < FR < 0.65
Heterozygous duplication		1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	Duplication	1.75 < FR < 2.15
Ambiguous copy number		All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *MECP2* and *ARX* genes. 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.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.
- Deletion of a probe's recognition sequence on the X-chromosome will lead to a complete absence of the corresponding probe amplification product in males, whereas female heterozygotes are recognizable by a 35-50% reduction in relative peak height.

P015 specific note:

- The use of fixed cut-off values for the FR of the probes as mentioned in the table above may not allow detection of mosaic deletions or duplications. Mosaic *CDKL5* deletions have been reported in CDKL5 deficiency disorder (Bartnik et al. 2011; Boutry-Kryza et al. 2014; Mei et al. 2014). In order to detect mosaic samples, the experiment has to have little variation and the final ratios should be significantly different from the reference samples (see Coffalyser.Net Reference Manual, Appendix I – Normalisation and result interpretation). Mosaic samples may not be detected if the percentage of cells that have the deletion or duplication is low.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *MECP2*, *CDKL5* and *ARX* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P015 MECP2.
- Not all exons of the *CDKL5*, *ARX* and *NTNG1* genes are covered in SALSA MLPA Probemix P015 MECP2. MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

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

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

Mutation databases

RettBASE for *MECP2* and *CDKL5* mutations: http://mecp2.chw.edu.au/mecp2/mecp2_home.php and http://mecp2.chw.edu.au/cdkl5/cdkl5_home.php. We strongly encourage users to deposit positive results in the RettBASE.

Leiden Open Variation Database (LOVD) for *ARX* and *NTNG1* mutations: https://databases.lovd.nl/shared/genes/ARX and https://databases.lovd.nl/shared/genes/NTNG1.

We strongly encourage users to deposit positive results in the LOVD. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *MECP2* exons 1 and 3 but not exon 2) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P015-F2 MECP2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*MECP2* Xq28	*CDKL5* Xp22.13	*ARX* Xp21.3	*NTNG1* 1p13.3
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 00797-L21056	5q				
137 « ¬ +	**GDI1 probe** 16875-L19669		Xq28			
144	**CDKL5 probe** 06466-L06567			**Exon 10**		
149	**NTNG1 probe** 18447-L24143					**Exon 2**
155 ~	**MECP2 probe** 18442-L24243		**Exon 4**			
161 « +	**ARX probe** 18440-L24338				**Exon 1**	
166	Reference probe 08222-L24146	10q				
172 « ¬ +	**SLC6A8 probe** 01879-L24147		Xq28			
178 « ¬	**IRAK1 probe** 10835-L24148		Xq28			
184 «	**MECP2 probe** 10836-L24339		**Exon 1**			
190	**CDKL5 probe** 06458-L23617			**Exon 3**		
196	**MECP2 probe** 10839-L23618		**Exon 3**			
202 «	**MECP2 probe** 03409-L16570		**Exon 1**			
208	Reference probe 09865-L08705	13q				
214 «	**MECP2 probe** 03770-L13387		**Upstream**			
221 ¬	**F8 probe** 06288-L05892		Xq28			
229	**MECP2 probe** 18441-L12494		**Exon 4**			
235 «	**ARX probe** 06455-L21229				**Exon 5**	
241 ¬	**L1CAM probe** 07051-L14553		Xq28			
247	Reference probe 05959-L05376	7p				
254 «	**MECP2 probe** 03768-L23833		**Exon 2**			
260 « ±	**MECP2 probe** 01769-L23834		**Exon 4**			
266	Reference probe 10728-L22588	6p				
274	**MECP2 probe** 01768-L13824		**Exon 4**			
280	Reference probe 16434-L24149	18q				
286	**CDKL5 probe** 06462-L24150			**Exon 6**		
292	**MECP2 probe** 18444-L24151		**Exon 4**			
299	Reference probe 16621-L24152	1q				
305 «	**MECP2 probe** 02002-L24153		**Exon 1**			
312	**NTNG1 probe** 06487-L24154					**Exon 5**
319 ¬	**DKC1 probe** 18761-L24340		Xq28			
338	Reference probe 09776-L24156	15q				
346 ~	**MECP2 probe** 01347-L24157		**Exon 4**			
356	**MECP2 probe** 10841-L24158		**Exon 4**			
365	**MECP2 probe** 01348-L24159		**Exon 3**			
373 «	**MECP2 probe** 01349-L24160		**Exon 2**			
384 ¬	**IDH3G probe** 01887-L23933		Xq28			
391	**MECP2 probe** 14737-L24161		**Exon 3**			
400	**CDKL5 probe** 06465-L05991			**Exon 9**		
409	Reference probe 17462-L21218	12p				
418 «	**MECP2 probe** 18446-L23620		**Exon 4**			
427	**NTNG1 probe** 06488-L23934					**Exon 6**
438	**NTNG1 probe** 06483-L24162					**Exon 3**
447 « ¬	**FLNA probe** 04138-L24163		Xq28			
457 ¬	**VAMP7 probe** 01094-L24164		Xq28 (**PAR2**)			
467	Reference probe 02674-L24165	11q				

[a] See section Exon numbering on page 4 for more information.

~ Probe located immediately adjacent to the deletion-prone region (DPR) in *MECP2*, in which copy number changes and SNVs occur frequently (Laccone et al. 2004; Huppke et al. 2005; Vidal et al. 2019).
± SNP rs267608346 could influence the 260 nt MECP2 probe signal. 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
+ The presence of salt in DNA samples can result in incomplete denaturation of CpG islands, which may result in false positive results: apparent deletions of this probe should be handled with care. Usually Coffalyser.Net issues a sample denaturation warning when the 88 nt and/or 96 nt D-fragments are too low. The 135 nt GDI, 161 nt ARX and 172 nt SLC6A8 probes target extremely GC-rich chromosomal areas, and are affected by salt concentrations that not yet affect the control D- fragments, thus without Coffalyser.Net issuing a warning. False positive results are more likely when DNA has been extracted by the Qiagen EZ1, M48 or M96 systems, as these leave a higher salt concentration in the sample. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration or other related technique).

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

Table 2. P015-F2 probes arranged according to chromosomal location

Table 2a. *MECP2* (Xq28)

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_004992.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		q-telomere			~90.0 kb
457 ¬	01094-L24164	*VAMP7* (**PAR2 region**)		TGTGGGAAAAGT-GTTTCCATTCTG	957.0 kb
221 ¬	06288-L05892	*F8*		TTCAGGGAGTCT-GGCCAAGGAAAA	218.1 kb
319 ¬	18761-L24340	*DKC1*		CATGATGTGCTT-GATGCTCAGTGG	331.9 kb
137 « ¬ +	16875-L19669	*GDI1*		CCTGACCATGGA-CGAGGAATACGA	79.7 kb
447 « ¬	04138-L24163	*FLNA*		TGACGGCACGTA-TACAGTGGCCTA	221.7 kb
		MECP2			
		start codon	*213-215 (Exon 2)*		
214 «	03770-L13387	Upstream	1.1 kb before exon 1	GCAAGAATGTTA-GTTTGCTGTCTG	0.7 kb
305 «	02002-L24153	Exon 1	394 nt before exon 1 reverse	GGGACGCCTGTT-TGCGCTGCTCTG	0.1 kb
202 «	03409-L16570	Exon 1	331 nt before exon 1	CATTAATCCTTA-ACATTCAAATTC	0.4 kb
184 «	10836-L24339	Exon 1	38-39	GGGCTGTGGTAA-AAGCCGTCCGGA	5.3 kb
254 «	03768-L23833	Exon 2	44 nt before exon 2	GAAAAAGGTCGT-GCAGCTCAATGG	0.1 kb
373 «	01349-L24160	Exon 2	174-175	GACTCCCCAGAA-TACACCTTGCTT	59.6 kb
196	10839-L23618	Exon 3	47 nt before exon 3	ACTTGTTCTGCA-GACTGGCATGTT	0.2 kb
365	01348-L24159	Exon 3	371-372	GCCCACCACTCT-GCTGAGCCCGCA	0.2 kb
391	14737-L24161	Exon 3	568-569	CTCTGCTGGGAA-GTATGATGTGTA	0.7 kb
356	10841-L24158	Exon 4 (4b)	32 nt before exon 4	AGAGCCTCTAAT-TGTTCCTTGTGT	0.3 kb
229	18441-L12494	Exon 4 (4b)	882-883	TCCTTGTCAAGA-TGCCTTTTCAAA	0.3 kb
346 ~	01347-L24157	Exon 4 (4b)	1229-1230	CTGAAGACCTGT-AAGAGCCCTGGG	0.2 kb
155 ~	18442-L24243	Exon 4 (4b)	1465-1466	AGAGGAGAAGAT-GCCCAGAGGAGG	0.1 kb
274	01768-L13824	Exon 4 (4b)	1608-1609	TTTCATCCTCCA-TGCCAAGGCCAA	0.1 kb
292	18444-L24151	Exon 4 (4b)	1668-1669	CCGAGAGAGTTA-GCTGACTTTACA	2.2 kb
418 «	18446-L23620	Exon 4 (4b)	3857-3858	TGCTGCCATGAA-CTGTCAAGTGTG	3.2 kb
260 « ±	01769-L23834	Exon 4 (4b)	7022-7023	CAGTAACACATA-GACTGTGCGCAT	5.9 kb
		stop codon	*1671-1673 (Exon 4)*		
178 « ¬	10835-L24148	*IRAK1*		TTTATGAAGCTT-TTCCAGGCTCCC	154.4 kb
241 ¬	07051-L14553	*L1CAM*		CAGCGGGTGAAA-ACTACAGTGTCG	70.3 kb
384 ¬	01887-L23933	*IDH3G*		TCCCCGAAACTT-CGCACCCCGTCG	99.8 kb
172 « ¬ # +	01879-L24147	*SLC6A8*		ACCCCGCTGGTC-TGCATGGTAAGG	

Table 2b. *CDKL5* (Xp22.13)

Length (nt)	SALSA MLPA probe	*CDKL5* exon[a]	Ligation site NM_003159.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*251-253 (Exon 2)*		
190	06458-L23617	Exon 3	41 nt before exon 3	GAGCTTTGTAGT-TTGTATGCGTGC	69.1 kb
286	06462-L24150	Exon 6	570-571	GCCAAATGGAGT-TCCACCTGAGAA	8.2 kb
400	06465-L05991	Exon 9	910-911	ATTGACCAACTT-TTTACTATTCAG	7.3 kb
144	06466-L06567	Exon 10	1023-1024	TCCTCAGTCCTT-GGAAAGAAGATA	
		stop codon	*3341-3343 (Exon 21)*		

Table 2c. *ARX* (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
		start codon	*229-231 (Exon 1)*		
161 « +	18440-L24338	Exon 1	115-116	AGATCGCAATAA-TATCCGTTATAA	11.2 kb
235 «	06455-L21229	Exon 5	1981-1982	CAGCACCACTCA-AGACCAAATGGA	
		stop codon	*1915-1917 (Exon 5)*		

Table 2d. *NTNG1* (1p13.3)

Length (nt)	SALSA MLPA probe	*NTNG1* exon[a]	Ligation site NM_014917.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*638-640 (Exon 2)*		
149	18447-L24143	Exon 2	408-409	ACAAGTATGTTA-GGCTTCCACCAA	176.5 kb
438	06483-L24162	Exon 3	1436-1437	GGATAAGGCTGT-TAAGACCAGCCG	83.1 kb
312	06487-L24154	Exon 5	227 nt after exon 5	TATGACTTTTCT-GACTACTCTTAA	72.7 kb
427	06488-L23934	Exon 6	1737-1738	GAATGTCTGCGA-CAACGAGCTCCT	
		stop codon	*1952-1954 (Exon 6)*		

[a] See section Exon numbering on page 4 for more information.
[b] 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 immediately adjacent to the deletion-prone region (DPR) in *MECP2*, in which copy number changes and SNVs occur frequently (Laccone et al. 2004; Huppke et al. 2005; Vidal et al. 2019).
± SNP rs267608346 could influence the 260 nt MECP2 probe signal. 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
+ The presence of salt in DNA samples can result in incomplete denaturation of CpG islands, which may result in false positive results: apparent deletions of this probe should be handled with care. Usually Coffalyser.Net issues a sample denaturation warning when the 88 nt and/or 96 nt D-fragments are too low. The 135 nt GDI, 161 nt ARX and 172 nt SLC6A8 probes target extremely GC-rich chromosomal areas, and are affected by salt concentrations that not yet affect the control D- fragments, thus without Coffalyser.Net issuing a warning. False positive results are more likely when DNA has been extracted by the Qiagen EZ1, M48 or M96 systems, as these leave a higher salt concentration in the sample. High salt concentrations can also be due to evaporation (dried out samples; SpeedVac concentration or other related technique).
# 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 tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P075 TCF4-FOXG1	Contains five probes for the *FOXG1* gene. The ligation sites of the FOXG1 probes are identical to those in P395 MECF2-FOXG1, with the exception of one of the exon 1 probes.
P106 X-linked ID	Contains three probes for the *ARX* gene. The ligation site of the ARX exon 1 probe is identical to the ARX exon 1 probe in P015 MECP2.
P137 SCN1A	Contains probes for the *SCN1A* gene. Mutations in *SCN1A* have been described in two females who fulfil the diagnostic criteria for classic RTT (Henriksen et al. 2018).
P189 CDKL5/ARX/FOXG1	Contains probes for the *CDKL5*, *NTNG1*, *ARX* and *FOXG1* genes.
P245 Microdeletion Syndromes-1A	Contains three probes for the *MECP2* gene. The ligation sites of these probes are identical to probes included in P015 MECP2.
P395 MEFC2-FOXG1	Contains five probes for the *FOXG1* gene. The ligation sites of the FOXG1 probes are identical to those in P075 TCF4-FOXG1, with the exception of one of the exon 1 probes.

References

- Amir RE et al. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet*. 23(2):185-188.
- Archer HL et al. (2006). Gross rearrangements of the MECP2 gene are found in both classical and atypical Rett syndrome patients. *J Med Genet*. 43(5):451-456.
- Bartnik M et al. (2011). Early-onset seizures due to mosaic exonic deletions of CDKL5 in a male and two females. *Genet Med.* 13(5):447-452.
- Borg I et al. (2005). Disruption of Netrin G1 by a balanced chromosome translocation in a girl with Rett syndrome. *Eur J Hum Genet*. 13(8):921-927.
- Boutry-Kryza N et al. (2014). Complex mosaic CDKL5 deletion with two distinct mutant alleles in a 4-year-old girl. *Am J Med Genet A.* 164A(8):2025-2028.
- Fehr S et al. (2013). The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy. *Eur J Hum Genet*. 21(3):266-273.
- Hagberg B et al. (1983). A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: Report of 35 cases. *Ann Neurol.* 14(4):471-479.
- Hagberg B et al. (2002). An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett Syndrome Clinical Criteria Consensus Panel Satellite to European Paediatric Neurology Society Meeting, Baden Baden, Germany, 11 September 2001. *Eur J Paediatr Neurol*. 6(5):293-297.
- Hagberg BA and Skjeldal OH. (1994). Rett variants: A suggested model for inclusion criteria. *Pediatr Neurol*. 11(1):5-11.
- Hardwick SA et al. (2007). Delineation of large deletions of the MECP2 gene in Rett syndrome patients, including a familial case with a male proband. *Eur J Hum Genet*. 15(12):1218-1229.
- Henriksen MW et al. (2018). De novo mutations in SCN1A are associated with classic Rett syndrome: a case report. *BMC Med Genet*. 19:184.
- Huppke P et al. (2005). Molecular diagnosis of Rett syndrome. *J Child Neurol*. 20(9):732-736.
- Jakimiec M et al. (2020). CDKL5 deficiency disorder - A complex epileptic encephalopathy. *Brain Sci*. 10(2):107.
- Kalscheuer VM et al. (2003). Disruption of the serine/threonine kinase 9 gene causes severe X-linked infantile spasms and mental retardation. *Am J Hum Genet*. 72(6):1401-1411.
- Kato M et al. (2004). Mutations of ARX are associated with striking pleiotropy and consistent genotype-phenotype correlation. *Hum Mutat*. 23(2):147-159.

- Laccone F et al. (2004). Large deletions of the MECP2 gene detected by gene dosage analysis in patients with Rett syndrome. *Hum Mutat*. 23(3):234-244.
- Mei D et al. (2014). Optimizing the molecular diagnosis of CDKL5 gene-related epileptic encephalopathy in boys. *Epilepsia.* 55(11):1748-1753.
- Neul JL et al. (2008). Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. *Neurology*. 70(16):1313-1321.
- Neul JL et al. (2010). Rett syndrome: Revised diagnostic criteria and nomenclature. *Ann Neurol.* 68(6):944-950.
- Pan H et al. (2006). Large deletions of the MECP2 gene in Chinese patients with classical Rett syndrome. *Clin Genet*. 70(5):418-419.
- Percy AK et al. (2007). Rett syndrome: North American database. *J Child Neurol*. 22(12):1338-1341.
- Philippe C et al. (2006). Spectrum and distribution of MECP2 mutations in 424 Rett syndrome patients: a molecular update. *Eur J Med Genet*. 49(1):9-18.
- Pini G et al. (2016). Rett syndrome: a wide clinical and autonomic picture. *Orphanet J Rare Dis.* 11:132.
- Scala E et al. (2005). CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. *J Med Genet*. 42(2):103-107.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Shoubridge C et al. (2010). ARX spectrum disorders: making inroads into the molecular pathology. *Hum Mutat*. 31(8):889-900.
- Stosser MB et al. (2018). High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. *Genet Med*. 20(4):403-410.
- 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.
- Wallerstein R et al. (2008). Expansion of the ARX spectrum. *Clin Neurol Neurosurg.* 110(6):631-634.
- Weaving LS et al. (2004). Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. *Am J Hum Genet*. 75(6):1079-1093.
- Zahorakova D et al. (2007). Mutation analysis of the MECP2 gene in patients of Slavic origin with Rett syndrome: novel mutations and polymorphisms. *J Hum Genet*. 52(4):342-348.

Selected publications using SALSA MLPA Probemix P015 MECP2

- Aradhya S et al. (2012). Exon-level array CGH in a large clinical cohort demonstrates increased sensitivity of diagnostic testing for Mendelian disorders. *Genet Med.* 14(6):594-603.
- Archer HL et al. (2006). Gross rearrangements of the MECP2 gene are found in both classical and atypical Rett syndrome patients. *J Med Genet*. 43(5):451-456.
- Bartholdi D et al. (2006). Clinical profiles of four patients with Rett syndrome carrying a novel exon 1 mutation or genomic rearrangement in the MECP2 gene. *Clin Genet*. 69(4):319-326.
- Bartnik M et al. (2011). Early-onset seizures due to mosaic exonic deletions of CDKL5 in a male and two females. *Genet Med*. 13(5):447-452.
- Bijlsma EK et al. (2012). Xq28 duplications including MECP2 in five females: Expanding the phenotype to severe mental retardation. *Eur J Med Genet*. 55(6-7):404-413.
- Echenne B et al. (2009). Neurologic aspects of MECP2 gene duplication in male patients. *Pediatr Neurol*. 41(3):187-191.
- Erlandson A et al. (2003). Multiplex ligation-dependent probe amplification (MLPA) detects large deletions in the MECP2 gene of Swedish Rett syndrome patients. *Genet Test*. 7(4):329-332.
- Fieremans N et al. (2014). De novo MECP2 duplications in two females with intellectual disability and unfavorable complete skewed X-inactivation. *Hum Genet*. 133(11):1359-1367.
- Friez MJ et al. (2006). Recurrent infections, hypotonia, and mental retardation caused by duplication of MECP2 and adjacent region in Xq28. *Pediatrics*. 118(6):e1687-1695.

- Mayo S et al. (2011). De novo interstitial triplication of MECP2 in a girl with neurodevelopmental disorder and random X chromosome inactivation. *Cytogenet Genome Res*. 135(2):93-101.
- Sharaf-Eldin WE et al. (2020). Mutation spectrum in the gene encoding methyl-CpG-binding protein 2 in Egyptian patients with Rett syndrome. *Meta Gene*. 24:100620.
- Zhang Q et al. (2019). Genomic mosaicism in the pathogenesis and inheritance of a Rett syndrome cohort. *Genet Med*. 21(6):1330-1338.

P015 product history	
Version	*Modification*
F2	Two reference probes have been replaced and the 118 nt Y fragment has been removed.
F1	Four MECP2 probes (exons 3 and 4) and two additional reference probes have been included. One ARX, one NTNG1 and several reference probes have been replaced. One CDKL5 and one IRAK1 probe have been removed. The 88 and 96 nt denaturation control probes have been replaced (QDX2).
E1	One MECP2 exon 3 probe has been removed.
D2	Several new MECP2 probes have been included and extra probes up- and downstream of *MECP2* have been added. In addition, probes for the *CDKL5*, *ARX* and *NTNG1* genes have been newly added, and all reference probes have been replaced.
D1	Test lot.
C2	Extra control fragments at 88, 96, 100 and 105 nt have been added.
B1	One extra probe for *MECP2* exon 1, three extra probes for *MECP2* exon 4 and extra reference probes have been added. Note that according to old product descriptions, lot 0504 to 0707 belong to a P015C version which is the same as the B1 version.
A1	First release.

Implemented changes in the product description

Version F2-07 – 16 November 2023 (04P)
- In section Reference samples, clarification added that all reference samples need to be the same sex for correct data analysis.
- Warning for hypersensitivity to salt added for the probes at 135 nt (GDI), 161 nt (ARX), and 172 nt (SLC6A8) in Table 1 and Table 2.
- Updated Rett syndrome clinical subgroups information and related references.
- Various minor textual or layout changes.
- Tables were renumbered.

Version F2-06 – 21 March 2023 (04P)
- Intended purpose adjusted; mutations in CDKL5 are associated with CDKL5 deficiency disorder.
- Clinical background section updated; section completely rewritten and information about CDKL5 deficiency disorder added.
- Performance characteristics section updated; percentage of cases explained by deletions or duplications adjusted and information about CDKL5 deficiency disorder added.
- Note about mosaicism added to the Interpretation of results section, P015 specific note.
- Probe remark about the deletion-prone / hotspot region in MECP2 adjusted below Table 2 and Table 3.
- Probe remark about SNPs adjusted below Table 2 and Table 3; information about rs201741317 and rs782037353 removed as their influence on the 427 nt NTNG1 and 447 nt FLNA probe signals, respectively, could not be verified.
- Probe remark for 319 nt DKC1 probe about location in or near a GC-rich region removed from Table 2 and Table 3a; probe sensitivity to salt contamination is within set limits.
- Information about related SALSA MLPA probemixes updated.
- Selected publications using SALSA MLPA Probemix P015 MECP2 updated.
- P015 product history adjusted; modifications in D2 and B1 versions clarified.
- Colombia added as country with IVD status.
- Various minor textual and layout changes.

Version F2-05 – 25 August 2021 (04P)

- References to SALSA MLPA Probemix P259 RPS6KA3 removed throughout the document as this probemix was discontinued in July 2021.
- References to SALSA MLPA Probemix P374 Microdeletion Syndromes 8 removed throughout the document as this probemix will be discontinued in October 2021.
- Small changes of probe lengths in Table 2 and 3 in order to better reflect the true lengths of the amplification products.
- Minor textual and layout changes throughout the document.

Version F2-04 – 16 March 2021 (04P)
- Product description rewritten and adapted to a new template.
- Updated the intended purpose and changed X-linked intellectual disability syndrome into early infantile epileptic encephalopathy.
- Various minor textual or layout changes.
- Positive DNA sample information is updated; six new samples added and NA23676 result is adjusted.
- Ligation sites of the probes targeting the MECP2, CDKL5, ARX and NTNG1 genes updated according to new version of the NM_ reference sequence.
- Warning added to Table 2 and Table 3a for 319 nt probe DKC1 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.
- Warning added to Table 2 and Table 3 for SNP rs201741317 that could influence the 427 nt NTNG1 probe signal and SNP rs782037353 that could influence the 448 nt FLNA probe signal.
- Exon numbering of the MECP2 gene has been changed.
- Added P137 SCN1A probemix to the Related SALSA MLPA probemixes list.
- Added references to the Reference list and updated the Selected publications list.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version F2-03 – 18 May 2020 (04)
- Israel added as country with IVD status.

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

	EUROPE*  ISRAEL COLOMBIA
	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.