

Product Description

SALSA® MLPA® Probemix P026-E2 Sotos

To be used with the MLPA General Protocol.

Version E2

As compared to version E1, one reference probe has been replaced and one reference probe has been removed. For complete product history see page 8.

Catalogue numbers:

- **P026-025R:** SALSA MLPA Probemix P026 Sotos, 25 reactions.
- **P026-050R:** SALSA MLPA Probemix P026 Sotos, 50 reactions.
- **P026-100R:** SALSA MLPA Probemix P026 Sotos, 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.

General information

The SALSA MLPA Probemix P026 Sotos is a **research use only (RUO)** assay for the detection of deletions or duplications in the *NSD1* and *NFIX* genes, which are associated with Sotos syndrome.

Sotos syndrome is a non-progressive neurological disorder with mental retardation, characterised by overgrowth and a distinctive craniofacial configuration. The syndrome is associated with a number of abnormalities: brain, cardiac, urogenital, musculoskeletal (scoliosis), ophthalmologic, dental and neoplastic. It is a genetic disorder due to haploinsufficiency of the *NSD1* gene (Nuclear receptor-binding SET Domain protein 1). A Sotos-like phenotype has also been associated with Malan syndrome (known as Sotos syndrome 2) which is caused by deletions of the *NFIX* gene. *NFIX* mutations distinctly cause Malan syndrome or Marshall-Smith syndrome, Malan syndrome being associated with amongst others whole gene deletions (Klaassens et al. 2015).

The *NSD1* gene (23 exons) spans ~166 kb of genomic DNA and is located on chromosome 5q35.3, ~177 Mb from the p-telomere. The *NFIX* gene (10 exons), spans ~103 kb of genomic DNA and is located on chromosome 19p13.13, ~13 Mb from the p-telomere.

More information is available at <https://www.ncbi.nlm.nih.gov/books/NBK1479/>.

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 *NSD1* exon numbering used in this P026-E2 Sotos product description is the exon numbering from the LRG_512 sequence. The *NFIX* exon numbering is the exon numbering from the NG_032925.2 sequence. 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 P026-E2 Sotos contains 46 MLPA probes with amplification products between 124 and 504 nucleotides (nt). This includes 25 probes for the *NSD1* gene: one probe for each exon, one additional probe for exon 12 and one probe upstream of exon 1. This also includes ten probes for the *NFIX* gene: one probe for each exon except exons 1 and 7 and two additional probes for introns 1 and 8. The intron probes detect the coding sequence of transcript variant 1 of the *NFIX* gene (see Table 2b). In addition, 11 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 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 different unrelated individuals who are from families without a history of Sotos syndrome. 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 NA01535 and NA04371 from the Coriell Institute have been tested with this P026-E2 probemix at MRC Holland and can be used as positive control samples to detect a heterozygous duplication of the *NSD1* gene. The quality of cell lines can change; therefore samples should be validated before use.

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 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 for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	$0.80 < FR < 1.20$
Homozygous deletion	FR = 0
Heterozygous deletion	$0.40 < FR < 0.65$
Heterozygous duplication	$1.30 < FR < 1.65$
Heterozygous triplication/homozygous duplication	$1.75 < FR < 2.15$
Ambiguous copy number	All other values

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

- 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. 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *NSD1* and *NFIX* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P026 Sotos.
- 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.

NSD1 and *NFIX* mutation databases

<https://databases.lovd.nl/shared/genes/NSD1> and <https://databases.lovd.nl/shared/genes/NFIX>. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 *NSD1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P026-E2 Sotos

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a		
		Reference	NSD1	NFIX
64-105	Control fragments – see table in probemix content section for more information			
124	Reference probe 15370-L13762	7q		
130	Reference probe 19616-L26704	4p		
136	NFIX probe 21167-L29461			Exon 2
142	NSD1 probe 02589-L11228		Exon 4	
148	NSD1 probe 02595-L02066		Exon 13	
154	Reference probe 02417-L01864	6p		
159	NSD1 probe 19346-L17204		Exon 16	
166	NSD1 probe 14897-L16639		Exon 12	
172	NFIX probe 21168-L29462			Exon 6
178	NSD1 probe 01301-L11231		Exon 23	
184	NSD1 probe 02590-L11232		Exon 7	
190 ∅	NFIX probe 21169-L29463			Intron 1
197 ∅	NFIX probe 21170-L29707			Intron 8
202 Ж	NFIX probe 21171-SP0994-L29465			Exon 5
211	NSD1 probe 01303-L00855		Exon 17	
219	NFIX probe 21172-L29466			Exon 4
227	NSD1 probe 17561-L00854		Exon 14	
238	NSD1 probe 02591-L02062		Exon 8	
244	Reference probe 15110-L16881	9q		
255 «	NSD1 probe 14898-L16640		Exon 1	
266 Ж	NSD1 probe 21173-SP0995-L29467		Exon 6	
274	NFIX probe 21174-L29468			Exon 3
283	NSD1 probe 15040-L16792		Exon 3	
292	NSD1 probe 02592-L17205		Exon 9	
301 «	NSD1 probe 15039-L16791		Exon 2	
310	Reference probe 15855-L17948	11p		
319	NSD1 probe 21175-L29469		Exon 11	
328	NFIX probe 21176-L29470			Exon 8
337 «	NSD1 probe 01310-L11225		upstream	
346	NSD1 probe 21138-L29708		Exon 15	
355	Reference probe 15508-L17363	13q		
364	NSD1 probe 02601-L02072		Exon 22	
377	NSD1 probe 15043-L16795		Exon 20	
383	Reference probe 00973-L13269	10q		
400	NSD1 probe 02598-L02069		Exon 18	
409	NFIX probe 21178-L29472			Exon 9
420 *	Reference probe 20900-L28954	1p		
427	NSD1 probe 02599-L17899		Exon 19	
436	NSD1 probe 21179-L29473		Exon 5	
445	NSD1 probe 02594-L11222		Exon 12	
454	NSD1 probe 02600-L02071		Exon 21	
463	NFIX probe 21180-L29474			Exon 10
472	Reference probe 18992-L24784	2q		
481	NSD1 probe 21182-L29476		Exon 10	
492	Reference probe 17001-L18577	20q		
504	Reference probe 18539-L23848	17q		

^a See section Exon numbering on page 2 for more information.

* New in version E2.

« 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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

∅ Intron probe. This probe detects the coding sequence of NM_001271043.2, which represents transcript variant 1 of the *NFIX* gene. This sequence has an alternative exon 1 and an additional coding exon located in intron 8, as compared to NM_002501.4.

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.

Table 2. P026-E2 probes arranged according to chromosomal location

Table 2a. *NSD1* gene

Length (nt)	SALSA MLPA probe	<i>NSD1</i> exon ^a	Ligation site NM_022455.5	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	198-200 (Exon 2)		
337 «	01310-L11225	upstream	768 nt before exon 1 <i>reverse</i>	ATGGAGGCAGCA-GCTCGGGTCTGC	1.3 kb
255 «	14898-L16640	Exon 1	366 nt after exon 1	ATATTGATGAGA-GCGATCGGCTCG	1.1 kb
301 «	15039-L16791	Exon 2	527-528	GCTCAGACGCCA-ATTGTTTGCCT	56.5 kb
283	15040-L16792	Exon 3	1155-1156	CTACGCCACTGA-AGTATGAAGTTG	12.3 kb
142	02589-L11228	Exon 4	1318-1319	AGATCCTTCTGA-GAGAGCCTGGGT	6.1 kb
436	21179-L29473	Exon 5	2081-2080 <i>reverse</i>	CGCTTTTCTCA-TCACCTGCTCCA	25.6 kb
266 Ж	21173-SP0995-L29467	Exon 6	4092-4091; 4038-4037 <i>reverse</i>	CTGTACCTTCTT-54 nt spanning oligo-CCACTTGCTTGG	2.4 kb
184	02590-L11232	Exon 7	4143-4144	GTGAAGAGGAAA-GCCTTCTAGCCC	1.5 kb
238	02591-L02062	Exon 8	4438-4439	GAAACTTCTTGA-ATCCAATGATTT	4.4 kb
292	02592-L17205	Exon 9	4511-4512	TGCTATGAAGCT-GGTCACCTGGAG	2.5 kb
481	21182-L29476	Exon 10	4581-4582	TCATAGGCACTA-CCAAGATATTTG	1.7 kb
319	21175-L29469	Exon 11	4829-4830	CTCAAGGAGAAT-GTCTGTCAGGTA	3.4 kb
445	02594-L11222	Exon 12	4872-4873	TGCTGTTATGTG-AGGCTCAGTGCT	0.1 kb
166	14897-L16639	Exon 12	4949-4950	TTTATCTGCAAT-GAATGTGCGACA	5.3 kb
148	02595-L02066	Exon 13	5076-5077	ACCCACCCACTG-TTATGCAGAACA	2.9 kb
227	17561-L00854	Exon 14	5210-5211	TACCACGCCAAT-GACTTTTGCCTG	7.6 kb
346	21138-L29708	Exon 15	5447-5448	GACTGTAAAGCA-GGCAAAAAGCCA	2.1 kb
159	19346-L17204	Exon 16	5668-5669	GAGCAGCAAGGA-TAAGATGGGCAA	4.0 kb
211	01303-L00855	Exon 17	5770-5771	AAGACAGCTGCA-GGAAGACCGAAA	7.0 kb
400	02598-L02069	Exon 18	6010-6011	GTGCTTTTCAA-GCGCCAATATCC	1.8 kb
427	02599-L17899	Exon 19	6168-6167 <i>reverse</i>	AGTGATATCATG-TTCTTGAGCATA	1.4 kb
377	15043-L16795	Exon 20	6332-6333	CTTTTTGCACTA-AGTGACATTAATA	5.0 kb
454	02600-L02071	Exon 21	6404-6405	GGAAAGACTGTT-TGCAAATGTGGA	3.2 kb
364	02601-L02072	Exon 22	6570-6571	GTTTTAGTTGTG-GGGATGCTGGCC	2.7 kb
178	01301-L11231	Exon 23	7615-7616	AGCTTCACCTCA-TCAGGTCACACC	
		<i>stop codon</i>	8286-8288 (Exon 23)		

Table 2b. *NFIX* gene

Length (nt)	SALSA MLPA probe	<i>NFIX</i> exon ^a	Ligation site NM_002501.4	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	364-366 (<i>Exon 1</i>)		
	No probe	Exon 1			
190 ∅	21169-L29463	Intron 1	386 nt before exon 2 (54-55 in NM_001271043.2)	TGCGATAGAACA-TGGAGATGTCAT	0.9 kb
136	21167-L29461	Exon 2	876-875 reverse	AGTTCCTTTGATT-GTGACTCCAATG	47.6 kb
274	21174-L29468	Exon 3	942-943	CAATCAGATAGT-TCAAACCAGCAA	0.4 kb
219	21172-L29466	Exon 4	1020-1021	TTTGTGACTTCC-GGGGTCTGGAAT	0.5 kb
202 Ж	21171-SP0994-L29465	Exon 5	1084-1085; 1112-1113	CAGCATCAGGGC-28 nt spanning oligo-TCCCAGCTACTA	1.7 kb
172	21168-L29462	Exon 6	1252-1253	TGTTCTATCCCG-GGACAGGCCGTT	6.1 kb
	No probe	Exon 7			
328	21176-L29470	Exon 8	1462-1461 reverse	GTGCAGGGCTGA-TGCTGTGGCCCG	6.4 kb
197 ∅	21170-L29707	Intron 8	2 kb before exon 9 (1423-1424 in NM_001271043.2)	TCCTATGCCTGA-TTCCAAATCCAC	2.3 kb
409	21178-L29472	Exon 9	1699-1700	GAACATCCCACA-GCAGTCTCAGGT	4.8 kb
463	21180-L29474	Exon 10	2221-2222	ATGATGGGGTTT-AGATCCCTCAGG	
		<i>stop codon</i>	1687-1689 (<i>Exon 9</i>)		

^a See section Exon numbering on page 2 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 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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

∅ Intron probe. This probe detects the coding sequence of NM_001271043.2, which represents transcript variant 1 of the *NFIX* gene. This sequence has an alternative exon 1 and an additional coding exon located in intron 8, as compared to NM_002501.4.

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

- ME030 BWS/RSS: Primary screening for Beckwith-Wiedemann syndrome and Silver-Russell syndrome.
- P018 SHOX: contains probes for the *SHOX* gene and several other probes in the PAR region.
- P216 GHD: contains probes for *GH1*, *PROP1*, *POU1F1*, *GHRHR*, *HESX1*, *LHX3* and *LHX4*.
- P217 IGF1R: contains probes for *IGF1R* and *IGFBP3*.
- P262 GHI: contains probes for *IGF1*, *GHR*, *JAK2* and *STAT5B*.

References

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- 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.
- 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 P026 Sotos

- Dikow N et al. (2013). The phenotypic spectrum of duplication 5q35. 2–q35. 3 encompassing NSD1: Is it really a reversed Sotos syndrome? *Am J Med Genet A*. 161:2158-2166.
- Douglas J et al. (2005). Partial NSD1 deletions cause 5% of Sotos syndrome and are readily identifiable by multiplex ligation dependent probe amplification. *J Med Genet*. 42:e56.
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- Vieira GH et al. (2015). Clinical and molecular heterogeneity in brazilian patients with sotos syndrome. *Mol Syndromol*. 6:32-38.

P026 product history	
Version	Modification
E2	One reference probe has been replaced and one reference probe has been removed.
E1	Ten new probes have been added for <i>NFIX</i> , five <i>NSD1</i> probes and ten reference probes have been replaced and one flanking probe has been removed.
D2	One flanking probe and one reference probe have been removed, four reference probes have been replaced and the control fragments have been adjusted (QDX2).
D1	Seven <i>NSD1</i> probes and six reference probes have been replaced. Two extra control fragments at 100 and 105 nt have been included.
C1	The 88 and 96 nt DNA denaturation control fragments (D-fragments) have been added. Several probes have a small change in length (+/- 1 nt).

Implemented changes in the product description
<p>Version E2-01 – 24 November 2020 (04P)</p> <ul style="list-style-type: none"> - 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). - Ligation sites of the probes targeting the <i>NSD1</i> and <i>NFIX</i> genes updated according to new versions of the NM_ reference sequences. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. <p>Version 11 – 08 March 2017 (55)</p> <ul style="list-style-type: none"> - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and 2, new picture included). - <i>NFIX</i> gene added in Table 1 and Table 2. - Changes of probe lengths in Table 1 and Table 2 in order to better reflect the true lengths of the amplification products. - Various textual and layout changes on page 1 and throughout the document.

More information: www.mrcholland.com ; www.mrcholland.eu	
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