Product Description
SALSA® MLPA® Probemix P318-B2 Hirschsprung-2

To be used with the MLPA General Protocol.

Version B2
As compared to version B1, five reference probes have been replaced. For complete product history see page 8.

Catalogue numbers:
- **P318-025R:** SALSA MLPA Probemix P318 Hirschsprung-2, 25 reactions.
- **P318-050R:** SALSA MLPA Probemix P318 Hirschsprung-2, 50 reactions.
- **P318-100R:** SALSA MLPA Probemix P318 Hirschsprung-2, 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 P318 Hirschsprung is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PHOX2B, GFRA3, GFRA2, GFRA1, EDNRB, NRTN, PSPN*, and *SOX10* genes, which are associated with Hirschsprung disease.

Hirschsprung disease is the main cause of functional intestinal obstruction. This disorder is characterised by the absence of the enteric ganglia along a variable length of the intestine. Two types of Hirschsprung disease have been described. The short-segment form, in which the aganglionic segment does not extend beyond the upper sigmoid, accounts for 80% of the cases. The long-segment form, in which aganglionosis extends proximal to the sigmoid, accounts for the remaining 20% of the cases. Both forms can be caused by dominant mutations in the *RET* gene (P169 Hirschsprung-1 probemix), as well as by recessive mutations in several other genes.

This P318 has been designed to detect deletions and duplications of one or more exons of eight genes involved in Hirschsprung disease: *PHOX2B*, *GFRA3*, *GFRA2*, *GFRA1*, *EDNRB*, *NRTN*, *PSPN*, and *SOX10*.

More information is available at https://www.ncbi.nlm.nih.gov/books/ and https://www.ncbi.nlm.nih.gov/omim?cmd=search

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

PHOX2B, GFRA3, GFRA2, GFRA1, EDNRB, NRTN, PSPN, and *SOX10* exon numbering used in this P318-B2 Hirschsprung-2 product description is the exon numbering from the LRG_513, NG_046894.1, NG_029215.2, NG_050620.1, NG_011630.3, NG_008202.1, NM_004158.5 and LRG_271 sequences. The *EDNRB* exon numbering has changed. From description version B2-01 onwards, we have adopted the NCBI exon numbering that is present in both the NG_011630.3 and NM_001122659.3 sequence for this gene. The exon numbering used in previous versions of this product description can be found in between brackets in Table 2. 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 and 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 P318-B2 Hirschsprung-2 contains 47 MLPA probes with amplification products between 130 and 472 nucleotides (nt). This includes: three probes for the *PHOX2B* gene; five probes for the *GFRA3* gene; five probes for the *GFRA2* gene; six probes for the *GFRA1* gene; eight probes for the *EDNRB* gene; three probes for the *NRTN* gene; three probes for the *PSPN* gene; five probes for the *SOX10* gene (see Table 2). In addition, nine 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 Hirschsprung disease. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples

MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The 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 *PHOX2B, GFRA3, GFRA2, GFRA1, EDNRB, NRTN, PSPN, and SOX10* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P318 Hirschsprung-2.
- 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.

LOVD mutation database

https://databases.lovd.nl/shared/genes/. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (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 *SOX10* exons 1 and 3 but not exon 2) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P318-B2 Hirschprung-2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]								
		Reference	*SOX10*	*PSPN*	*NRTN*	*EDNRB*	*GFRA1*	*GFRA2*	*GFRA3*	*PHOX2B*
64-105	Control fragments – see table in probemix content section for more information									
130 *	Reference probe 20879-L29296	12q								
136	**SOX10 probe** 11174-L11858		**Exon 3**							
142	**GFRA1 probe** 19948-L27009						**Exon 8**			
148 «	**NRTN probe** 11158-L11842				**Exon 1**					
154	**EDNRB probe** 11135-L11819					**Exon 1**				
164	**GFRA3 probe** 14389-L11841								**Exon 8**	
169 *	Reference probe 20384-L25345	21q								
173 «	**PHOX2B probe** 11167-L30319									**Exon 3**
178	**EDNRB probe** 11137-L11821					**Exon 3**				
184 *	Reference probe 18809-L24305	1p								
188	**GFRA2 probe** 11148-L30394							**Exon 4**		
195	**EDNRB probe** 14387-L16374					**Exon 7**				
202	**GFRA1 probe** 11142-L11826						**Exon 2**			
209	**EDNRB probe** 11139-L11823					**Exon 6**				
217	**GFRA3 probe** 14388-L16375								**Exon 4**	
226	**SOX10 probe** 11176-L16376		**Exon 4**							
234	**GFRA3 probe** 11156-L16377								**Exon 6**	
241 «	**NRTN probe** 11163-L16378				**Exon 2**					
247	**EDNRB probe** 11138-L16379					**Exon 5**				
256	**SOX10 probe** 11175-L16380		**Exon 4**							
262	Reference probe 05959-L30320	7p								
267	**EDNRB probe** 14385-L30321					**Exon 2**				
274 «	**NRTN probe** 11161-L16070				**Exon 2**					
281	Reference probe 21259-L29623	15q								
288	**EDNRB probe** 11140-L30324					**Exon 7**				
294	**GFRA2 probe** 14711-L30325							**Exon 6**		
303 *	Reference probe 11929-L11124	16p								
310	**SOX10 probe** 14383-L11857		**Exon 2**							
319	**GFRA1 probe** 14713-L16385						**Exon 5**			
328	**PSPN probe** 11168-L16382			**Exon 1**						
337	**GFRA2 probe** 14712-L16384							**Exon 7**		
344	Reference probe 08741-L30322	9q								
350	**GFRA1 probe** 19887-L30323						**Exon 10**			
355	**GFRA2 probe** 11147-L11831							**Exon 3**		
364	**PSPN probe** 11170-L16381			**Exon 2**						
373 «	**PHOX2B probe** 11164-L11848									**Exon 1**
382	**SOX10 probe** 14715-L16387		**Exon 1**							
391	**PSPN probe** 11169-L11853			**Exon 1**						
400	**GFRA1 probe** 11143-L16069						**Exon 3**			
409	**GFRA2 probe** 11151-L11835							**Exon 9**		
418	**EDNRB probe** 14382-L11818					**Exon 1**				
427	**GFRA3 probe** 14716-L16388								**Exon 3**	
436	**GFRA3 probe** 11152-L11836								**Exon 2**	
445 «	**PHOX2B probe** 11166-L11850									**Exon 2**
454	**GFRA1 probe** 11144-L11828						**Exon 6**			
463 *	Reference probe 16106-L18276	17q								
472	Reference probe 14846-L16554	3q								

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

* New in version B2.
« 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.

Table 2. P318-B2 probes arranged according to chromosomal location

Tabel 2a. *PHOX2B* gene

Length (nt)	SALSA MLPA probe	*PHOX2B* Exon[a]	Ligation site NM_003924.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*116-118 (exon 1)*		
373 «	11164-L11848	Exon 1	9-10	AGGAGGCCAGTT-TGGAGGCAGGCG	1.3 kb
445 «	11166-L11850	Exon 2	448-449	GCCCAGCTCAAA-GAGCTGGAAAGG	2.8 kb
173 «	11167-L30319	Exon 3	2250-2251	GATGGACTGTAT-ATTGAGGGTTCC	
		Stop Codon	*1058-1060 (exon 3)*		

Tabel 2b. *GFRA3* gene

Length (nt)	SALSA MLPA probe	*GFRA3* Exon[a]	Ligation site NM_001496.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*198-200 (exon 1)*		
436	11152-L11836	Exon 2	409-410	CACCTCTAGCAT-AAGCACCCCACT	4.8 kb
427	14716-L16388	Exon 3	630-631	CCAGCAAACCCT-GGAAAATGAATC	1.7 kb
217	14388-L16375	Exon 4	704-705	GCCATGCTGTGT-ACTCTCAATGAC	4.1 kb
234	11156-L16377	Exon 6	1187-1188	GAGTGTGAAATG-CTGGAAGGGTTC	1.0 kb
164	14389-L11841	Exon 8	1531-1532	AAGCTAAGGGTT-ATGACCTCCAGA	
		Stop Codon	*1398-1400 (exon 8)*		

Table 2c. *GFRA2* gene

Length (nt)	SALSA MLPA probe	*GFRA2* Exon[a]	Ligation site NM_001495.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*717-719 (exon 1)*		
355	11147-L11831	Exon 3	1145-1146	AGGCTTGCTTCA-ATCTTCTCAGGT	35.8 kb
188	11148-L30394	Exon 4	1234-1235	CCTGAATGACAA-CTGCAAGAAGCT	45.8 kb
294	14711-L30325	Exon 6	1651-1652	CTATGTGGACTC-CAGCCCCACTGG	2.3 kb
337	14712-L16384	Exon 7	1893-1894	GTGACAGTACCA-GCTTGGGGACCA	9.5 kb
409	11151-L11835	Exon 9	2016-2017	TCCCAGGGAGTA-ACAAGGTGATCA	
		Stop Codon	*2109-2111 (exon 9)*		

Table 2d. *GFRA1* gene

Length (nt)	SALSA MLPA probe	*GFRA1* Exon[a]	Ligation site NM_005264.8	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*291-293 (exon 2)*		
202	11142-L11826	Exon 2	151-152	GAGTTTCCTCTT-TCACTGGATGGA	1.1 kb
400	11143-L16069	Exon 3	402-403	ATCAGTGCCTGA-AGGAGCAGAGCT	59.4 kb
319	14713-L16385	Exon 5	709-710	TTTCTTGGCAGA-TGTTTTTCAGCA	86.3 kb
454	11144-L11828	Exon 6	927-928	ACGGAATGCTCT-TCTGCTCCTGCC	31.6 kb
142	19948-L27009	Exon 8	1196-1197	CCCAACTACATA-GACTCCAGTAGC	28.2 kb
350	19887-L30323	Exon 10	1501-1502	ACAGAAGCTGAA-ATCCAATGTGTC	
		Stop Codon	*1686-1688 (exon 11)*		

Table 2e. *EDNRB* gene

Length (nt)	SALSA MLPA probe	*EDNRB* Exon[a]	Ligation site NM_001122659.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*259-261 (exon 1)*		
418	14382-L11818	Exon 1 (2)	392-393	AACCGCAGAGAT-AATGACGCCACC	0.3 kb
154	11135-L11819	Exon 1 (2)	672-673	GGTCCCAATATC-TTGATCGCCAGC	14.6 kb
267	14385-L30321	Exon 2 (3)	763-764	AGGACTGGCCAT-TTGGAGCTGAGA	0.4 kb
178	11137-L11821	Exon 3 (4)	1024-1025	TGCGAATCTGCT-TGCTTCATCCCG	2.5 kb
247	11138-L16379	Exon 5 (6)	1226-1227	GGAAGTGGCCAA-AACCGTCTTTTG	0.7 kb
209	11139-L11823	Exon 6 (7)	1364-1365	GGTATTGGACTA-TATTGGTATCAA	2.8 kb
288	11140-L30324	Exon 7 (8)	2656-2657	GTCCACATGACA-AAGGGGCAGGTA	0.4 kb
195	14387-L16374	Exon 7 (8)	3020-3021	AGGAAATGAGGT-GGGGTTGGAGGA	
		Stop Codon	*1585-1587 (exon 7)*		

Table 2f. *NRTN* gene

Length (nt)	SALSA MLPA probe	*NRTN* Exon[a]	Ligation site NM_004558.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*784-786 (exon 1)*		
148 «	11158-L11842	Exon 1	677-678	GGCAGGCGTTCA-AAGTCAAAGGCC	3.9 kb
274 «	11161-L16070	Exon 2	1149-1150	TACGCGTCCGAC-GAGACGGTGCTG	0.2 kb
241 «	11163-L16378	Exon 2	1340-1341	CTACCACACGGT-GCACGAGCTGTC	
		Stop Codon	*1375-1377 (exon 2)*		

Table 2g. *PSPN* gene

Length (nt)	SALSA MLPA probe	*PSPN* Exon[a]	Ligation site NM_004158.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*85-87 (exon 1)*		
328	11168-L16382	Exon 1	93-94	ACAATGGCCGTA-GGGAAGTTCCTG	0.1 kb
391	11169-L11853	Exon 1	196-197	GAGAGTTCTCGT-CTGAACAGGTGG	0.2 kb
364	11170-L16381	Exon 2	333-334	CTGGGCTACGCC-TCAGAGGAGAAG	
		Stop Codon	*553-555 (exon 2)*		

Table 2h. *SOX10* gene

Length (nt)	SALSA MLPA probe	*SOX10* Exon[a]	Ligation site NM_006941.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*302-304 (exon 2)*		
382	14715-L16387	Exon 1	184-185	CACTTCCTAAGG-ACGAGCCCCAGA	0.8 kb
310	14383-L11857	Exon 2	503-504	ACGATGACAAGT-TCCCCGTGTGCA	5.5 kb
136	11174-L11858	Exon 3	785-786	AGCGGCTCCGTA-TGCAGCACAAGA	4.0 kb
256	11175-L16380	Exon 4	1156-1157	AGCCACGAGGTA-ATGTCCAACATG	1.3 kb
226	11176-L16376	Exon 4	2464-2465	AATCAGAGACAA-TTCACAGAGCCT	
		Stop Codon	*1700-1702 (exon 4)*		

[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.

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.

References

- 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.

Related SALSA MLPA probemixes

- P169 Hirschsprung-1: Contains probes for the RET, ZEB2, EDN3 and GDNF genes, involved in Hirschsprung disease.

Selected publications using SALSA MLPA Probemix P318 Hirschsprung-2

- Wang G et al. (2021). Analysis of genotype–phenotype relationships in 90 Chinese probands with Waardenburg syndrome. Hum Gen. doi:10.1007/s00439-021-02301-3.

P318 product history	
Version	*Modification*
B2	Five reference probes have been replaced.
B1	Nine reference probes are added and the length of several probes adjusted.
A2	The control fragments have been changed (QDX2) and some lengths have been adjusted.
A1	First release.

Implemented changes in the product description

Version B2-01 – 14 October 2021 (04P)
- Product description restructured and adapted to a new template.
- Exon numbering of the *EDNRB* gene has been changed according to NG_011630.3, on page 7.
- Ligation sites of the probes targeting the *PHOX2B*, *GFRA3*, *GFRA2*, *GFRA1*, *NRTN*, *PSPN* and *SOX10* genes updated according to new version of the NM_ reference sequence.
- GC-rich region warning removed for the *SOX10* gene

Version 06 – 28 September 2017 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Ligation sites of the probes targeting the GFRA1, EDNRB, NRTN, and PSPN genes updated according to new version of the NM_reference sequences.
- Data analysis method has been modified.
- Various minor textual changes.

Version 05 – 31 August 2017 (55)
- Warning added in Tables 1 and 2, 136 nt probe 11174-L11858, 148 nt probe 11158-L11842, 172 nt probe 11167-L16372, 226 nt probe 11176-L16376, 240 nt probe 11163-L16378, 256 nt probe 11175-L16380, 274 nt probe 11161-L16070, 310 nt probe 14383-L11857, 373 nt probe 11164-L11848, 382 nt probe 14715-L16387, and 445 nt probe 11166-L11850.

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