Product Description
SALSA® MLPA® Probemix P185-C3 Intersex

To be used with the MLPA General Protocol.

Version C3
For complete product history see page 9.

Catalogue numbers:
- **P185-025R:** SALSA MLPA Probemix P185 Intersex, 25 reactions.
- **P185-050R:** SALSA MLPA Probemix P185 Intersex, 50 reactions.
- **P185-100R:** SALSA MLPA Probemix P185 Intersex, 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 P185 Intersex is a **research use only (RUO)** assay for the detection of deletions or duplications in the *NR0B1* (*DAX1*), *SOX9*, *SRY*, *WNT4* and *NR5A1* genes.

The sex-determining region on chromosome Y (*SRY*) is the most important sex-determining region in humans. As a transcriptional activator, the SRY protein's main function is to initiate male sex determination by regulating a genetic switch in male development.

In addition to *SRY*, the dosage-sensitive sex reversal (DSS) gene, *NR0B1* (also known as *DAX1*) has also been found to influence sex-determination. Duplication of the *NR0B1* gene region, resulting in two active copies can override the testis-determining signal, resulting in the development of ovaries and an XY female. In testicular Sertoli and Leydig cells, *NR0B1* can be up-regulated by *WNT4*.

The *SOX9* gene functions as a critical Sertoli cell differentiation factor. *SOX9* deletions, as well as chromosomal rearrangements such as deletions in an enhancer-containing region far upstream of *SOX9* can lead to campomelic dysplasia with or without XY sex reversal (Leipoldt et al. 2007; Katoh-Fukui et al. 2015).

The *NR5A1* gene encodes the orphan nuclear receptor steroidogenic factor-1 (SF1) which plays a key role in regulating adrenal and gonadal development, steroidogenesis and reproduction. Haploinsufficiency of *NR5A1* has been described in several 46, XY individuals with mild gonadal dysgenesis and impaired androgenization, but normal adrenal function, suggesting that dosage-sensitive or domain-specific effects of SF1 action are important in human testicular development and function.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1547/.

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 *NR0B1* exon numbering used in this P185-C3 Intersex product description is the exon numbering from the LRG_858 sequence. The *WNT4* exon numbering used in this P185-C3 Intersex product description is the exon numbering from the NG_008974.1 sequence. The *NR5A1* exon numbering used in this P185-C3 Intersex product description is the exon numbering from the NG_008176.1 sequence. The *SOX9* exon numbering used in this P185-C3 Intersex product description is the exon numbering from the NG_012490.1 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 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 P185-C3 Intersex contains 45 MLPA probes with amplification products between 121 and 495 nucleotides (nt). This includes probes for the following genes: *NR0B1* (*DAX1*) and *CXorf21* (*TASL*) on Xp21.2, *SOX9* on 17q24.3, *SRY*, *UTY* and *ZFY* on Yp11.21-31, *WNT4* on 1p36.12 and *NR5A1* on 9q33.3. Furthermore, flanking probes specific for the X chromosome are included. 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 with the same sex chromosome copy number is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

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

Reference samples

A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of sex-determining region. To facilitate interpretation of results for probes targeting the X and Y chromosomes, it is recommended to use **XY male** reference samples. 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.

For sex chromosomes we recommend using XY male reference samples for easier interpretation of results. Theoretically expected ratios with corresponding copy numbers have been given in the table below for selected chromosomal anomalies when **XY male reference samples** have been used:

Theoretically expected ratios with corresponding copy numbers for selected sex chromosome anomalies normalized against XY male reference samples							
Targeted region		**X chromosome**		**Y chromosome**		**Autosomal chromosomes**	
		Ratio	Copy number	Ratio	Copy number	Ratio	Copy number
	46,XX	2*	2	0	0	1	2
	46,XY	1	1	1	1	1	2
Sex chromosome anomalies	**45,X0**	1	1	0	0	1	2
	47,XXX	3	3	0	0	1	2
	47,XXY	2	2	1	1	1	2
	47,XYY	1	1	2	2	1	2
	48,XXXY	3	3	1	1	1	2
	48,XXYY	2	2	2	2	1	2
	48,XYYY	1	1	3	3	1	2

* Note: in an XX female individual normalised against XY male reference samples ratio 2 (2 copies) is a normal result. In Coffalyser.Net software this is displayed as an aberrant result as it is different from the reference samples.

- 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 *WNT4* and *NR5A1* 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.

P185 specific note:

- MLPA cannot distinguish between normal females (46,XX) and triploid females (69,XXX).

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *NR0B1*, *CXorf21*, *SOX9*, *SRY*, *ZFY*, *WNT4* and *NR5A1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P185 Intersex.
- 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

https://databases.lovd.nl/shared/genes/NR5A1,
https://databases.lovd.nl/shared/genes/NR0B1,
https://databases.lovd.nl/shared/genes/SOX9,
https://databases.lovd.nl/shared/genes/WNT4 and
https://databases.lovd.nl/shared/genes/SRY.

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 *NR5A1* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P185-C3 Intersex

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*SOX9*	Chr Y	*NR0B1*	*WNT4*	*NR5A1*
64-105	Control fragments – see table in probemix content section for more information						
121 ¥	**ZFY probe** S0135-L27687			Yp11.31			
130	Reference probe 00797-L00463	5q					
136 #	**SOX9 probe** 07156-L06795		**Exon 1**				
142	Reference probe 02956-L02388	7q					
148	**NR0B1 probe** 06048-L05503				**Exon 1**		
154	Reference probe 15163-L16938	3q					
165	**SOX9 probe** 06051-L05506		**Exon 1**				
171 «	**NR5A1 probe** 16876-L20237						**Exon 2**
178	Reference probe 14329-L15998	6p					
187 «	**WNT4 probe** 06054-L19826					**Exon 1**	
196 ¬	**PHEX probe** 20664-L08286				Xp22.11		
202	**SOX9 probe** 16990-L28791		upstream				
214	**UTY probe** 11812-L12607			Yq11.21			
221	**SOX9 probe** 09604-L12188		upstream				
228 ¬	**COL4A5 probe** 20665-L28792				Xq22.3		
234	**NR0B1 probe** 06049-L12569				**Exon 1**		
241	**UTY probe** 19335-L28790			Yq11.21			
247	Reference probe 02869-L02336	1p					
256	**SOX9 probe** 06052-L05507		**Exon 2**				
265	Reference probe 14385-L11820	13q					
274	**WNT4 probe** 07159-L06798					**Exon 4**	
283 «	**NR5A1 probe** 16992-L20001						**Exon 3**
292 «	**WNT4 probe** 06055-L05510					**Exon 2**	
301	**SOX9 probe** 11501-L09981		upstream				
310	**NR0B1 probe** 06050-L05505				**Exon 2**		
319	**WNT4 probe** 16991-L20000					**Exon 5**	
328 « Ж	**NR5A1 probe** 16993-SP0424-L20002						**Exon 5**
337	**SOX9 probe** 06053-L05508		**Exon 3**				
343 «	**NR5A1 probe** 09673-L12239						**Exon 1**
351	**SRY probe** 01191-L19672			Yp11.31			
364	Reference probe 04357-L03777	7q					
373 Ж	**SOX9 probe** 16994-SP0425-L20003		upstream				
382	**SOX9 probe** 09607-L09980		upstream				
390 « Ж	**NR5A1 probe** 16995-SP0426-L20004						**Exon 6**
400	**WNT4 probe** 07158-L06797					**Exon 3**	
408 «	**NR5A1 probe** 09675-L10047						**Exon 4**
418	**NR5A1 probe** 16996-L12240						**Exon 7**
427 ¬	**GJB1 probe** 06188-L02097				Xq13.1		
436 ¬	**PQBP1 probe** 07886-L07700				Xp11.23		
445	Reference probe 08793-L08817	10q					
454 ¬	**NR0B1 probe** 14797-L16506				centromeric		
463 ¬	**CXorf21 probe** 14798-L16507				centromeric		
474 ¬	**CXorf21 probe** 14799-L16508				centromeric		
481 ¬	**NR0B1 probe** 14800-L16509				centromeric		
495	Reference probe 06676-L15676	11p					

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

¥ Changed in version C3. Minor alteration, no change in sequence detected.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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.

¬ 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.

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. P185-C3 probes arranged according to chromosomal location

Table 2a. *NR0B1* gene and other chromosome X probes

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Ligation site NM_000475.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
228 ¬	20665-L28792	*COL4A5*		CTTGCTTCAACT-GCATTGGAACTG	37.4 **M**b
427 ¬	06188-L02097	*GJB1*		ACAAGGTCCACA-TCTCAGGGACAC	21.7 **M**b
436 ¬	07886-L07700	*PQBP1*		GCTCTCCCCACA-TGACCCCAACTC	18.1 **M**b
474 ¬	14799-L16508	*CXorf21*	19.4 kb before *CXorf21*	TGCCATCACAAT-AATTAGTCGTCT	19.7 kb
463 ¬	14798-L16507	*CXorf21*	NM_025159.3; 131 nt after exon 1 *reverse*	TCTGATATGGTA-AGCTAGAAAAGC	141.9 kb
481 ¬	14800-L16509	*NR0B1*	126 kb upstream of *NR0B1*	TTAGCTCAAATC-AAAGCTCCTAAC	108.6 kb
454 ¬	14797-L16506	*NR0B1*	18 kb upstream of *NR0B1*	CCACATTTTCTG-TAGCTAATTTAT	17.7 kb
		start codon	*28-30 (Exon 1)*		
148	06048-L05503	*NR0B1* exon 1	2-3	GCCACTGGGCAG-AACTGGGCTACG	1.0 kb
234	06049-L12569	*NR0B1* exon 1	954-955	TTCGAGACTGTG-GAAGTCTCGGAG	3.7 kb
310	06050-L05505	*NR0B1* exon 2	1315-1316	TCATCGAACTTA-ATAGTACCCTTT	8.1 **M**b
		stop codon	*1438-1440 (Exon 2)*		
196 ¬	20664-L08286	*PHEX*		CTCGCAAGTATT-TAGCACAGTCTG	

Table 2b. Chromosome Y probes

Length (nt)	SALSA MLPA probe	Gene	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
351	01191-L19672	*SRY*	NM_003140.3; 570-571	CAACAGGTTGTA-CAGGGATGACTG	174.1 kb
121	S0135-L27687	*ZFY*	NM_003411.4; 646-647	TCATAGAGGAGG-ATGTTCAGTGCT	11.2 **M**b
241	19335-L28790	*UTY*	NM_007125.4; 860-861	CTTCGGTAGCTT-AAGTCTTTGCCT	124.6 kb
214	11812-L12607	*UTY*	127 kb upstream of *UTY*	AGGATCCTGGAT-ATTCCACTACCA	

Table 2c. *WNT4* gene

Length (nt)	SALSA MLPA probe	*WNT4* exon[a]	Ligation site NM_030761.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*176-178 (Exon 1)*		
187 «	06054-L19826	Exon 1	246-245 *reverse*	CTTACAGCCAGT-TGCTCGCGGCGG	13.1 kb
292 «	06055-L05510	Exon 2	314-315	GCGAGAAACTCA-AGGGCCTGATCC	8.3 kb
400	07158-L06797	Exon 3	581-582	AGCTGGAGAAGT-GCGGCTGTGACA	0.2 kb
274	07159-L06798	Exon 4	701-702	TGCGGGAGAGAA-GCAAGGGGGCCT	2.8 kb
319	16991-L20000	Exon 5	2848-2849	GACATGCATGGA-TCAAGACCTTGC	
		stop codon	*1229-1231 (Exon 5)*		

Table 2d. *NR5A1* gene

Length (nt)	SALSA MLPA probe	*NR5A1* exon[a]	Ligation site NM_004959.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*167-169 (Exon 2)*		
343 «	09673-L12239	Exon 1	149-150	GCTGCTTCCGCT-TCGTAAGTGAGG	3.9 kb
171 «	16876-L20237	Exon 2	171-170 *reverse*	CGTACGAATAGT-CCATGCCCGCGG	0.2 kb
283 «	16992-L20001	Exon 3	302-303	AGAACAACAAGC-ACTACACGTGCA	3.1 kb
408 «	09675-L10047	Exon 4	983-984	CCGACCAGACCT-TCATCTCCATCG	7.0 kb
328 « Ж	16993-SP0424-L20002	Exon 5	1060-1061; 1086-1087	ATGACGCTGCTG-26 nt spanning oligo-GTTCGACCACAT	2.0 kb
390 « Ж	16995-SP0426-L20004	Exon 6	1298-1299; 21 nt after exon 6	TCATCCTCTTCA-27 nt spanning oligo-GAGAGGTGGAGA	8.2 kb
418	16996-L12240	Exon 7	1477-1478	AAGGAGTACCTG-TACCACAAGCAC	
		stop codon	*1550-1552 (Exon 7)*		

Table 2e. *SOX9* gene

Length (nt)	SALSA MLPA probe	*SOX9* exon[a]	Ligation site NM_000346.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
221 +	09604-L12188	1500 kb before exon 1		CGTGTCAGAAAT-ATCCAGTGAGAA	0.4 kb
202 +	16990-L28791	1500 kb before exon 1		CAGTCTAGGGCT-GCTGTTTTATTG	492.5 kb
382 +	09607-L09980	1007 kb before exon 1		AAGGGCCATCAT-TCTGGACAGTGC	523.8 kb
301 +	11501-L09981	483 kb before exon 1		CCTGCAGGACAA-ATCTGATCACTG	242.5 kb
373 Ж +	16994-SP0425-L20003	241 kb before exon 1		TTCTGGCAGCTA-31 nt spanning oligo-GGTCCTCCCTTA	240.8 kb
		start codon	*373-375 (Exon 1)*		
165	06051-L05506	Exon 1	35-36	AACTGACTGGAA-ACTTCAGTGGCG	0.7 kb
136 #	07156-L06795	Exon 1	760-759 *reverse*	GGCGTTGTGCAA-GTGCGGGTACTG	1.1 kb
256	06052-L05507	Exon 2	985-986	ACGCCATCTTCA-AGGCGCTGCAGG	2.5 kb
337	06053-L05508	Exon 3	2954-2955	AGGCAGATGCCT-GCTCGCTCTGTC	
		stop codon	*1900-1902 (Exon 3)*		

[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.

¬ 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.

+ Deletions and other rearrangements in the upstream region of *SOX9* have been described as having a similar effect as *SOX9* deletions. This is due to the presence of strong *SOX9* enhancer sequences in this region. We have no information on the effects of a copy number change of a single probe in this region.

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. Single probe aberration(s) must be confirmed by another method.

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P185 product history	
Version	*Modification*
C3	One probe was adjusted in length.
C2	The lengths of several probes have been adjusted.
C1	Probes for *CYP21A2* have been removed and probes for the *CXorf21* gene as well as additional probes for *NR0B1*, *NR5A1* and the Y chromosome have been included. The number of reference probes has been increased from 6 to 9.
B2	The 88 and 96 nt control fragments have been replaced (QDX2); the 100 & 105 nt control fragments have been added.
B1	Several new probes have been added for the *NR5A1* gene and the region upstream of the *SOX9* gene.
A1	First release.

Implemented changes in the product description
Version C3-02 – 05 July 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the CXorf21, NR0B1, NR5A1, SOX9, SRY, WNT4 and ZFY genes updated according to new version of the NM_ reference sequence. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - Interpretation of results section clarified with additional text and table. - Missing remark symbols for salt sensitivity added to Table 2d. Version C3-01 – 18 July 2019 (02P) - Product description restructured and adapted to a new template. - Product description adapted to a new product version. - Small changes in Table 1 and Table 2. - 105 nt Y control fragment has been removed from Table 2b.

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