

Product Description SALSA[®] MLPA[®] Probemix P185-C3 Intersex

To be used with the MLPA General Protocol.

Version C3. As compared to version C2, one probe was adjusted in length. 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.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 *NROB1* (*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, *NROB1* (also known as *DAX1*) has also been found to influence sex-determination. Duplication of the *NROB1* 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, *NROB1* 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 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 exon numbering used in this P185-C3 Intersex product description are: the exon numbering from the RefSeq transcript NM_000475.4 for *NROB1*, exon numbering for *UTY* is the exon numbering from the RefSeq transcript NM_007125.4, RefSeq transcript NM_030761.4 for *WNT4*, RefSeq

transcript NM_004959.4 for *NR5A1*, RefSeq transcript NM_000346.3 for *SOX9*. The exon numbering and NM sequence used have been retrieved in July 2019. As changes to the NCBI database can occur after release of this product description, 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 nt. This includes probes for the following genes: *NROB1* (DAX1) and *CXorf21* on Xp21.2, *SOX9* on 17q24.3, *SRY*, *UTY* and *ZFY* on Yp11.31, *WNT4* on 1p36.12 and *NR5A1* on 9q33.3. Furthermore, flanking probes specific for the X and Y chromosomes are included. In addition, nine reference probes are included in this probemix, detecting several autosomal chromosomal locations.

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, one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment 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.mlpa.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 from peripheral blood, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥ 3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of sex-determining region. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (<https://catalog.coriell.org>) and DSMZ (<https://www.dsmz.de/home.html>) have a diverse collection of biological resources which may be used as a positive control DNA sample 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.mlpa.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 dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	$0.80 < DQ < 1.20$
Homozygous deletion	$DQ = 0$
Heterozygous deletion	$0.40 < DQ < 0.65$
Heterozygous duplication	$1.30 < DQ < 1.65$
Heterozygous triplication/Homozygous duplication	$1.75 < DQ < 2.15$
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *NR0B1*, *CXorf21*, *SOX9*, *SRY*, *ZFY*, *WNT4* and *NR5A1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P185-C3.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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/SOX9>;

<https://databases.lovd.nl/shared/genes/SRY>.

<https://databases.lovd.nl/shared/genes/NR0B1>;

<https://databases.lovd.nl/shared/genes/WNT4>;

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 SNPs and unusual results (e.g., a duplication of *NR5A1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P185-C3 Intersex

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a				
		Reference	SOX9	Chr X/Y	NR0B1	WNT4
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	5q31				
136	SOX9 probe 07156-L06795	Exon 1				
142	Reference probe 02956-L02388	7q31				
148	NR0B1 probe 06048-L05503	Exon 1				
154	Reference probe 15163-L16938	3q27				
165	SOX9 probe 06051-L05506	Exon 1				
171 ‹	NR5A1 probe 16876-L20237	Exon 2				
178	Reference probe 14329-L15998	6p22				
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	1p21				
256	SOX9 probe 06052-L05507	Exon 2				
265	Reference probe 14385-L11820	13q22				
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	7q21				
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	10q21				
454 ⇈	NR0B1 probe 14797-L16506	NR0B1 region				
463 ⇈	CXorf21 probe 14798-L16507	NR0B1 region				
474 ⇈	CXorf21 probe 14799-L16508	NR0B1 region				
481 ⇈	NR0B1 probe 14800-L16509	NR0B1 region				
495	Reference probe 06676-L15676	11p15				

a) See above section on exon numbering for more information.

† Changed in version C3 (from lot C3-0519 onwards). Small change in length, no change in sequence detected.

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.

‹ 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.

Table 2. P185-C3 probes arranged according to chromosomal location

Table 2a. *NROB1* gene and other chromosome X probes

Length (nt)	SALSA MLPA probe	Gene / Exon ^a	Ligation site NM_000475.4	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
228 ↪	20665-L28792	<i>COL4A5</i>		CTTGCTTCAACT-GCATTGGAACCTG	37365 kb
427 ↪	06188-L02097	<i>GJB1</i>		ACAAGGTCCACA-TCTCAGGGACAC	21716 kb
436 ↪	07886-L07700	<i>PQBP1</i>		GCTCTCCCCACA-TGACCCCAACTC	18119 kb
474 ↪	14799-L16508	<i>CXorf21</i>	19.4 kb before <i>CXorf21</i>	TGCCATCACAAT-AATTAGTCGTCT	19.7 kb
463 ↪	14798-L16507	<i>CXorf21</i>	NM_025159.2; 131 nt after exon 1, reverse	TCTGATATGGTA-AGCTAGAAAAGC	141.9 kb
481 ↪	14800-L16509	<i>NROB1</i>	126 kb upstream <i>NROB1</i>	TTAGCTCAAATC-AAAGCTCCTAAC	108.6 kb
454 ↪	14797-L16506	<i>NROB1</i>	18 kb upstream <i>NROB1</i>	CCACATTTCTG-TAGCTAATTTAT	17.7 kb
		<i>Start Codon</i>	16-18 (exon 1)		
148	06048-L05503	<i>NROB1</i> Exon 1	10 nt before exon 1	GCCACTGGGCAG-AACTGGGCTACG	1.0 kb
234	06049-L12569	<i>NROB1</i> Exon 1	942-943	TTCGAGACTGTG-GAAGTCTCGGAG	3.7 kb
310	06050-L05505	<i>NROB1</i> Exon 2	1303-1304	TCATCGAACTTA-ATAGTACCCTTT	8126 kb
		<i>Stop Codon</i>	1426-1428 (exon 2)		
196 ↪	20664-L08286	<i>PHEX</i>		CTCGCAAGTATT-TAGCACAGTCTG	

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

↪ 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.

Table 2b. Chromosome Y probes

Length (nt)	SALSA MLPA probe	Gene/Exon ^a	Ligation site NM_007125.4	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
351	01191-L19672	<i>SRY</i>	639-640 in NM_003140.2	CAACAGGTTGTA-CAGGGATGACTG	174.1 kb
121 ¥	S0135-L27687	<i>ZFY</i>	550-551 in NM_003411.3	TCATAGAGGAGG-ATGTTCACTGCT	11211.8 kb
241	19335-L28790	<i>UTY</i>	860-861	CTTCGGTAGCTT-AAGTCTTTGCCT	124.6 kb
214	11812-L12607	<i>UTY</i>	127 kb upstream of <i>UTY</i>	AGGATCTGGAT-ATTCCACTACCA	

¥ Changed in version C3 (from lot C3-0519 onwards). Small change in length, no change in sequence detected.

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 2c. *WNT4* gene

Length (nt)	SALSA MLPA probe	<i>WNT4</i> Exon ^a	Ligation site NM_030761.4	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		<i>Start Codon</i>	105-107 (exon 1)		
187 <	06054-L19826	Exon 1	175-174 reverse	CTTACAGCCAGT-TGCTCGCGGCGG	13.1 kb
292 <	06055-L05510	Exon 2	243-244	GCGAGAACTCA-AGGGCCTGATCC	8.3 kb
400	07158-L06797	Exon 3	510-511	AGCTGGAGAAGT-GCGGCTGTGACA	0.2 kb
274	07159-L06798	Exon 4	630-631	TGCGGGAGAGAA-GCAAGGGGGCCT	2.8 kb
319	16991-L20000	Exon 5	2777-2778	GACATGCATGGA-TCAAGACCTTGC	
		<i>Stop Codon</i>	1158-1160 (exon 5)		

<< 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.

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 2d. *NR5A1* gene

Length (nt)	SALSA MLPA probe	<i>NR5A1</i> Exon ^a	Ligation site NM_004959.4	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		<i>Start Codon</i>	188-190 (exon 2)		
343 <	09673-L12239	Exon 1	170-171	GCTGCTCCGCT-TCGTAAGTGAGG	3.9 kb
171 <	16876-L20237	Exon 2	192-191 reverse	CGTACGAATAGT-CCATGCCCGCGG	0.2 kb
283 <	16992-L20001	Exon 3	323-324	AGAACAACAAGC-ACTACACGTGCA	3.1 kb
408 <	09675-L10047	Exon 4	1004-1005	CCGACCAGACCT-TCATCTCCATCG	7.0 kb
328 Ж	16993-SP0424-L20002	Exon 5	1081-1082; 1107-1108	ATGACGCTGCTG-26 nt spanning oligo -GTTCCGACCACAT	2.0 kb
390 Ж	16995-SP0426-L20004	Exon 6	1319-1320; 21 nt after exon 6	TCATCCTCTTCA-27 nt spanning oligo -GAGAGGTGGAGA	8.2 kb
418	16996-L12240	Exon 7	1498-1499	AAGGAGTACCTG-TACCACAAGCAC	
		<i>Stop Codon</i>	1571-1573 (exon 7)		

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.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.

Table 2e. *SOX9* gene

Length (nt)	SALSA MLPA probe	<i>SOX9</i> Exon ^a	Ligation site NM_000346.3	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
		<i>Start Codon</i>	373-375 (exon 1)		
165	06051-L05506	Exon 1	35-36	AACTGACTGGAA-ACTTCAGTGCGG	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	
		<i>Stop Codon</i>	1900-1902 (exon 3)		

✂ 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.

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.

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

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.

Selected publications using SALSA MLPA Probemix P185-C3 Intersex

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- Laino L et al (2014). Disorders of sex development: a genetic study of patients in a multidisciplinary clinic. *Endocr Connect.* 2014 Dec;3(4):180-92.
- Phadke ND et al (2013). Detection of carrier status and mutations in family members of a child with complete deletion of the DAX1 gene using multiplex ligation-dependent probe amplification. *Indian J Pediatr.* Aug;80(8):704-5.
- Alfonsi M et al (2013). De novo 9q33 microdeletion identified by array-comparative genomic hybridization in a foetus with sex reversal and congenital heart defects. *Clin Dysmorphol.* 2013 Jul;22(3):132-4.
- Alves C et al (2010). 46,XX male - testicular disorder of sexual differentiation (DSD): hormonal, molecular and cytogenetic studies. *Arq Bras Endocrinol Metabol.* Nov;54(8):685-9.
- Soardi FC et al (2010). Complete XY gonadal dysgenesis due to p.D293N homozygous mutation in the NR5A1 gene: a case study. *J Appl Genet.* 51(2):223-4.
- Lecointre C et al (2009). Familial acampomelic form of campomelic dysplasia caused by a 960 kb deletion upstream of SOX9. *Am J Med Genet A.* Jun;149A(6):1183-9.

P185 Product history	
Version	Modification
C3	One probe was adjusted in length.
C2	The lengths of several probes have been adjusted.
C1	Probes for <i>CYP21A2</i> have been removed and probes for the <i>Cxorf21</i> gene as well as additional probes for <i>NR0B1</i> , <i>NR5A1</i> 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 <i>NR5A1</i> gene and the region upstream of the <i>SOX9</i> gene.
A1	First release.

Implemented changes in the product description
<p><i>Version C3-01 – 18 July 2019 (02P)</i></p> <ul style="list-style-type: none"> - 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. <p><i>Version 10 – 18 July 2016 (55)</i></p> <ul style="list-style-type: none"> - New references added on page 2. - Various minor textual changes. <p><i>Version 09 – 24 November 2015 (55)</i></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 Table 2, new picture included). <p><i>Version 08 – 07 May 2015 (54)</i></p> <ul style="list-style-type: none"> - New references added on page 2. - Electropherogram pictures using the old MLPA buffer removed. - Various minor textual changes.

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