Product Description
SALSA® MLPA® Probemix P311-B2 CHD

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 8.

Catalogue numbers:
- **P311-025R:** SALSA MLPA Probemix P311 CHD, 25 reactions.
- **P311-050R:** SALSA MLPA Probemix P311 CHD, 50 reactions.
- **P311-100R:** SALSA MLPA Probemix P311 CHD, 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 P311 CHD is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GATA4*, *NKX2-5*, *TBX5*, *BMP4,* and *CRELD1* genes and of the 22q11.21 region (DiGeorge region), which are associated with congenital heart disease (CHD).

CHD is a common birth defect, of which ventricular septal defects are collectively the most common type. Abnormal cardiac development originates from both environmental and genetic factors. Multiple studies postulate that mutations in several genes could be implicated in CHD.

The transcription factor GATA4 forms a complex with TBX5 which interacts with a heart muscle protein, α-myosin heavy chain. Another factor, the homeobox (developmental) gene, *NKX2-5* also interacts with *MYH6*. Mutations of all these proteins are associated with both atrial and ventricular septal defects. In addition, *NKX2-5* is associated with defects in the electrical conduction of the heart and *TBX5* is related to the Holt-Oram syndrome which includes electrical conduction defects and abnormalities of the upper limb. Atrioventricular septal defect (AVSD) can also be caused by mutations in the gene encoding cell adhesion molecule *CRELD1*. Bone morphogenetic protein 4 (BMP4) was shown to have a critical role in functional heart formation in model animals; the loss of this protein resulted in various developmental defects.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK500252.

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 *GATA4*, *NKX2-5*, *TBX5*, *BMP4,* and *CRELD1* exon numbering used in this P311-B2 CHD product description is the exon numbering from the NG_008177.2, LRG_671, LRG_670, NG_009215.1, and NG_017069.1 sequences, respectively. 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 P311-B2 CHD contains 41 MLPA probes with amplification products between 124 and 475 nucleotides (nt). This includes eight probes for the *GATA4* gene, four probes for the *NKX2-5* gene, ten probes for the *TBX5* gene, four probes for the *BMP4* gene, two probes for the *CRELD1* gene, and three probes for the 22q11.21 region (DiGeorge region). 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 congenital heart 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 *GATA4*, *NKX2-5*, *TBX5*, *BMP4,* and *CRELD1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P311 CHD.
- 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.

CHD-RF-KB mutation database

http://www.sysbio.org.cn/CHDRFKB. We strongly encourage users to deposit positive results in the Congenital Heart Disease associated Risk Factors KnowledgeBase (CHD-RF-KB). 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 *GATA4* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P311-B2 CHD

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]						
		Reference	*GATA4*	*TBX5*	*NKX2-5*	*22q11*	*BMP4*	*CRELD1*
64-105	Control fragments – see table in probemix content section for more information							
124	Reference probe 18709-L21056	5q						
130 ¬	**CTSB probe** 01212-L00766		**Downstream**					
136 «	**CRELD1 probe** 02141-L01620							**Exon 4**
142	**GATA4 probe** 07641-L07326		**Exon 5**					
148 «	**GATA4 probe** 08309-L08282		**Exon 1**					
154	Reference probe 21307-L29713	7q						
160	**TBX5 probe** 05694-L05136			**Exon 8**				
166	**TBX5 probe** 06207-L05127			**Exon 1**				
174	**TBX5 probe** 05696-L05138			**Exon 9**				
184	**TBX5 probe** 05687-L05129			**Exon 2**				
190	**NKX2**-5 **probe** 12465-L13480				**Exon 1**			
195	**TBX5 probe** 05688-L05130			**Exon 3**				
202	**GATA4 probe** 07643-L07328		**Exon 7**					
208 «	**GP1BB probe** 05464-L10114					**22q11**		
215	Reference probe 08570-L08571	17q						
229 « ¬	**GATA4 probe** 07697-L07414		**Upstream**					
238	**GATA4 probe** 07642-L07327		**Exon 6**					
247	**TBX5 probe** 05691-L05133			**Exon 6**				
254 «	**BMP4 probe** 12467-L14521						**Exon 1**	
266	**NKX2-5 probe** 11629-L12386				**Exon 2**			
274	**NKX2-5 probe** 12468-L13483				**Exon 1**			
283	**TBX5 probe** 05695-L05137			**Exon 8**				
292	Reference probe 11087-L11770	2p						
303	**TBX5 probe** 05697-L05139			**Exon 9**				
317 Ø	**TBX5 probe** 05686-L05128			**Intron 1**				
328	Reference probe 10682-L11264	6p						
337	**GATA4 probe** 07639-L07324		**Exon 3**					
346	**TBX5 probe** 06209-L05132			**Exon 5**				
355	**GATA4 probe** 07640-L07325		**Exon 4**					
362 « ¬	**GATA4 probe** 07696-L07413		**Upstream**					
369	Reference probe 12377-L13386	2q						
382 «	**DGCR8 probe** 08476-L10765					**22q11**		
391 «	**BMP4 probe** 12469-L13484						**Exon 4**	
408 «	**CRELD1 probe** 12470-L31541							**Exon 11**
418	Reference probe 11008-L11679	4q						
427	**NKX2-5 probe** 12471-L13486				**Exon 2**			
436 « Ø	**BMP4 probe** 12472-L13487						**Intron 1**	
445	Reference probe 15733-L17713	1p						
454 «	**BMP4 probe** 12473-L13488						**Exon 3**	
466	**CDC45 probe** 05463-L05808					**22q11**		
475	Reference probe 18501-L23726	19q						

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

« 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.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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. P311-B2 probes arranged according to chromosomal location

Table 2a. *GATA4*

Length (nt)	SALSA MLPA probe	*GATA4* exon[a]	Ligation site NM_002052.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
362 « ¬	07696-L07413	Upstream	8.4 kb upstream of exon 1	CATGCTCAAGAT-AGGCACTGGAGC	1.5 kb
229 « ¬	07697-L07414	Upstream	6.9 kb upstream of exon 1	GAGGTTCTTCTT-TAAAATCCATTC	7.0 kb
		start codon	*561-563 (Exon 2)*		
148 «	08309-L08282	Exon 1	22 nt after exon 1	TTTCTTCCCTTT-CTTTGCTCCTTC	44.6 kb
	No probe	Exon 2			
337	07639-L07324	Exon 3	1180-1181	CTCAGTAGATAT-GTTTGACGACTT	1.3 kb
355	07640-L07325	Exon 4	1462-1463	CTACATGAAGCT-CCACGGGGTACG	4.9 kb
142	07641-L07326	Exon 5	1538-1539	AAGAACCTGAAT-AAATCTAAGACA	1.9 kb
238	07642-L07327	Exon 6	1615-1616	CAACTCCAGCAA-CGCCACCACCAG	1.4 kb
202	07643-L07328	Exon 7	1791-1792	CACAAGGCTATG-CGTCTCCCGTCA	89.3 kb
		stop codon	*1887-1889 (Exon 7)*		
130 ¬	01212-L00766	*CTSB* gene	88 kb downstream of exon 7	AAGTGTAGCAAG-ATCTGTGAGCCT	

Table 2b. *NKX2-5*

Length (nt)	SALSA MLPA probe	*NKX2-5* exon[a]	Ligation site NM_004387.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*124-126 (Exon 1)*		
274	12468-L13483	Exon 1	369-370	CCGGCCAAGTGT-GCGTCTGCCTTT	0.1 kb
190	12465-L13480	Exon 1	10 nt after exon 1	AGGTGAGGAGGA-AACACAGGCCCC	1.6 kb
427 #	12471-L13486	Exon 2	576-575, reverse	CGCTCCAGCTCA-TAGACCTGCGCC	0.5 kb
266	11629-L12386	Exon 2	1052-1053	CGGGATTCCGCA-GAGCAACTCGGG	
		stop codon	*1096-1098 (Exon 2)*		

Table 2c. *TBX5*

Length (nt)	SALSA MLPA probe	*TBX5* exon[a]	Ligation site NM_000192.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*668-670 (Exon 2)*		
166	06207-L05127	Exon 1	509-510	GACGTTGGAAGA-AGACCTGGCCTA	2.2 kb
317 Ø	05686-L05128	Intron 1	1807 nt before exon 2	CTATTCTGGGTA-AGCAGTAAACCC	1.9 kb
184	05687-L05129	Exon 2	726-727	GCCTGACGCAAA-AGACCTGCCCTG	1.9 kb
195	05688-L05130	Exon 3	837-838	AATCAAAGTGTT-TCTCCATGAAAG	3.3 kb
	No probe	Exon 4			
346	06209-L05132	Exon 5	1138-1139	TCCTTCCAGAAA-CTCAAGCTCACC	3.8 kb
247	05691-L05133	Exon 6	1213-1214	TACCAGCCTAGA-TTACACATCGTG	28.5 kb
	No probe	Exon 7			
160	05694-L05136	Exon 8	1465-1466	GTGAGGCAAAAA-GTGGCCTCCAAC	0.2 kb
283	05695-L05137	Exon 8	1641-1642	CCATTGTACCAA-GAGGAAAGGTGA	10.1 kb
174	05696-L05138	Exon 9	1724-1725	AAGAAGATTCCT-TCTACCGCTCTA	1.1 kb
303	05697-L05139	Exon 9	2869-2870	TTTGCTTTGGTT-TTGTCCTGCCTT	
		stop codon	*2222-2224 (Exon 9)*		

Table 2d. *BMP4*

Length (nt)	SALSA MLPA probe	*BMP4* exon[a]	Ligation site NM_001202.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*409-411 (Exon 3)*		
254 «	12467-L14521	Exon 1	274-275	TGCAGGGACCTA-TGGTGAGCAAGG	2.1 kb
436 « Ø	12472-L13487	Intron 1	1054 nt before exon 2	CGCAGGCCGAAA-GCTGTTCACCGT	2.3 kb
	No probe	Exon 2			
454 «	12473-L13488	Exon 3	428-429	TGGTAACCGAAT-GCTGATGGTCGT	1.3 kb
391 «	12469-L13484	Exon 4	790-791	AACATCTGGAGA-ACATCCCAGGGA	
		stop codon	*1633-1635 (Exon 4)*		

Table 2e. *CRELD1*

Length (nt)	SALSA MLPA probe	*CRELD1* exon[a]	Ligation site NM_001031717.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*107-109 (Exon 2)*		
136 «	02141-L01620	Exon 4	414-415	CAAGTCAGACTT-CGAGTGCCACCG	6.3 kb
408 «	12470-L31541	Exon 11	1168-1169	GCATTCCCCATC-TTAACTGATTTA	
		stop codon	*1373-1375 (Exon 12)*		

Table 2f. 22q11 region (DiGeorge)

Length (nt)	SALSA MLPA probe	22q11 gene	Ligation site, region	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
466	05463-L05808	*CDC45*	NM_001178010.2; 155-156, region A-B	ATGTTCGTGTCC-GATTTCCGCAAA	244.0 kb
208 «	05464-L10114	*GP1BB*	NM_000407.5; 210-211, region A-B	CACAACCGAGCT-GGTGCTGACCGG	386.3 kb
382 «	08476-L10765	*DGCR8*	NM_022720.7; 2984-2985, region A-B	GACTCAGCGACT-GCACCAGTGGCA	

[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.

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.

¬ 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.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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

Related SALSA MLPA probemixes

P250 DiGeorge	Contains probes for the 22q11 DiGeorge region.
P234 GATA3 - GATA4	Contains probes for the *GATA3* and *GATA4* genes.

References

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- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
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Selected publications using SALSA MLPA Probemix P311 CHD

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- El Malti R et al. (2016). A systematic variant screening in familial cases of congenital heart defects demonstrates the usefulness of molecular genetics in this field. *Eur J Hum Genet*. 24(2), 228-236.
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- Moldovan V & Moldovan E (2020). Multiplex ligation-dependent probe amplification–a short overview. *Rev Romana de Medicina de Lab*, 28(2), 123-131.
- Mutlu ET et al. (2018). Analysis of gene copy number variations in patients with congenital heart disease using multiplex ligation-dependent probe amplification. *Anatol J Cardiol*. 20(01):9-15.
- Nagy O et al. (2019). Copy number variants detection by microarray and multiplex ligation-dependent probe amplification in congenital heart diseases. *J Biotechnol*, 299, 86-95.

P311 product history	
Version	*Modification*
B2	Two reference probes have been replaced, one reference probe has been removed and one probe length has been adjusted.
B1	One target and one flanking probe have been removed and two reference probes have been replaced.
A2	Control fragments have been adjusted (QDX2). One CRELD1 probe has been removed.
A1	First release.

Implemented changes in the product description

Version B2-02 – 24 May 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting the *NKX2-5*, *BMP4*, *CRELD1*, and *DGCR8* genes updated according to new version 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.
- Warning added to Table 2b for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

- Symbols added for flanking and Intronic probes in Table 1 and 2.
- Additional publications using SALSA MLPA Probemix P311 CHD added.

Version B2-01– 24 April 2019 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version.
- Small changes in Table 1, and changes in Table 2.

Version 07 (55) – 29 July 2016
- 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).
- Various minor textual changes on page 1.
- Exon numbering has changed in table 1 and 2 for genes NKX2-5, TBX5, BMP4 and CRELD1.
- Various minor layout changes

Version 06 (48) – 07 August 2015
- Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed.

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