Product Description SALSA® MLPA® Probemix P100-C2 MYBPC3

To be used with the MLPA General Protocol.

Version C2. As compared to version C1, five reference probes have been replaced and two probe lengths have been adjusted. For complete product history see page 6.

Catalogue numbers:
- **P100-025R:** SALSA MLPA Probemix P100 MYBPC3, 25 reactions.
- **P100-050R:** SALSA MLPA Probemix P100 MYBPC3, 50 reactions.
- **P100-100R:** SALSA MLPA Probemix P100 MYBPC3, 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 P100 MYBPC3 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MYBPC3* gene, which is associated with familial hypertrophic cardiomyopathy syndrome.

The *MYBPC3* gene (35 exons) spans ~21.3 kb of genomic DNA and is located on chromosome 11p11.2. The protein encoded by *MYBPC3* is the cardiac isoform of myosin-binding protein C (cMyBP-C), which is exclusively expressed in human heart muscle during development. The cMyBP-C protein is crucial for sarcomere organisation in cardiac muscle tissue and for maintenance of normal cardiac function. Defects in the *MYBPC3* gene account for approximately 20-40% of the cases of familial hypertrophic cardiomyopathy.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1768/.

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 *MYBPC3* exon numbering used in this P100-C2 MYBPC3 product description is the exon numbering from the RefSeq transcript NM_000256.3, which is identical to the LRG_386 sequence. The exon numbering and NM_ sequence used have been retrieved on 01/2020. 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 P100-C2 MYBPC3 contains 43 MLPA probes with amplification products between 130 and 454 nucleotides (nt). This includes 29 probes for the *MYBPC3* gene, one probe for each exon with the exception of exons 8, 10, 11, 14, 16, and 20, and five flanking probes located upstream and downstream of the *MYBPC3* gene (Table 2). These flanking probes can be useful to estimate the extent of deletions/duplications in the *MYBPC3* gene. In addition, nine reference probes are included and detect nine different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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.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 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 familial hypertrophic cardiomyopathy. 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 Institute (https://catalog.coriell.org) and Leibniz Institute 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

Copy number status	Dosage quotient
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. 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 *MYBPC3* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P100 MYBPC3.
- 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.

***MYBPC3* mutation database:** https://databases.lovd.nl/shared/genes/MYBPC3. 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 *MYBPC3* exons 4 and 6 but not exon 5) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P100-C2 MYBPC3

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*MYBPC3*	Flanking probes
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L00463	5q31		
136 ¬	**MADD probe** 08908-L08999			3.0 kb downstream
142 ¬	**SPI1 probe** 08911-L09002			2.3 kb upstream
148	**MYBPC3 probe** 08902-L11418		**Exon 17**	
153	**MYBPC3 probe** 17122-L20305		**Exon 3**	
160	**MYBPC3 probe** 08901-L08992		**Exon 15**	
166 *	Reference probe 22048-L31008	1p36		
175 «	**MYBPC3 probe** 03151-L02592		**Exon 1**	
184 ±	**MYBPC3 probe** 03158-L02599		**Exon 22**	
190 ¬	**PTPRJ probe** 05918-L05363			775 kb upstream
196 *	Reference probe 16259-L18551	3p21		
202	**MYBPC3 probe** 10803-L11443		**Exon 2**	
211	**MYBPC3 probe** 03159-L02600		**Exon 25**	
220 *	Reference probe 17867-L22126	19q13		
227	**MYBPC3 probe** 17130-L20313		**Exon 34**	
232	**MYBPC3 probe** 10804-L20709		**Exon 6**	
238	**MYBPC3 probe** 03160-L02601		**Exon 27**	
244	**MYBPC3 probe** 17124-L20307		**Exon 7**	
250 ¬	**SPI1 probe** 08910-L20710			2.9 kb upstream
257	**MYBPC3 probe** 03154-L20711		**Exon 9**	
265	**MYBPC3 probe** 10805-L11445		**Exon 29**	
274	**MYBPC3 probe** 17128-L20311		**Exon 30**	
280 ¥ ¬	**MADD probe** 20994-L32069			1.7 kb downstream
288 *	Reference probe 15880-L30312	2p16		
294	**MYBPC3 probe** 03155-L20757		**Exon 12**	
301	**MYBPC3 probe** 03162-L20702		**Exon 33**	
309 ¥	**MYBPC3 probe** 20995-L32070		**Exon 13**	
322	**MYBPC3 probe** 03163-L20753		**Exon 35**	
328	**MYBPC3 probe** 08905-L11421		**Exon 24**	
337	**MYBPC3 probe** 03959-L20754		**Exon 19**	
346	Reference probe 02473-L01917	15q21		
355	**MYBPC3 probe** 17126-L20309		**Exon 21**	
364	**MYBPC3 probe** 08907-L20703		**Exon 31**	
372	**MYBPC3 probe** 08904-L20704		**Exon 23**	
382	**MYBPC3 probe** 08900-L20705		**Exon 5**	
391 *	Reference probe 06672-L27891	10p15		
400	**MYBPC3 probe** 08903-L20707		**Exon 18**	
409	**MYBPC3 probe** 08906-L20708		**Exon 26**	
418	Reference probe 07442-L09394	9p24		
427	**MYBPC3 probe** 17123-L20306		**Exon 4**	
436	**MYBPC3 probe** 17127-L20310		**Exon 28**	
445	**MYBPC3 probe** 17129-L20312		**Exon 32**	
454	Reference probe 08274-L08153	8q24		

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

* New in version C2.
¥ Changed in version C2. Minor alteration, no change in sequence detected.

± A SNP could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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. *MYBPC3* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*MYBPC3* exon[a]	Ligation site NM_000256.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
190 ¬	05918-L05363	*PTPRJ* exon 7		GGGGAGACAGAT-TCTTCCAATCTC	772.4 kb
250 ¬	08910-L20710	*SPI1* exon 5		GATCCGCCTGTA-CCAGTTCCTGTT	0.6 kb
142 ¬	08911-L09002	*SPI1* exon 5		CTCAAGTCCGTA-TGTAAATCAGAT	2.3 kb
		start codon	*56-58 (Exon 1)*		
175 «	03151-L02592	Exon 1	53-54	TGACGTCTCTCA-GGATGCCTGAGC	1.4 kb
202	10803-L11443	Exon 2	4 nt after exon 2	AGAGGCAGGTAA-GATCCTGATCAG	0.6 kb
153	17122-L20305	Exon 3	355-356	CCAGAGAAGGCA-GAGCCCATGCTG	0.5 kb
427	17123-L20306	Exon 4	476-477	CAAGCTCAGCAG-CTCTCAATGGTC	0.3 kb
382	08900-L20705	Exon 5	648-649	CAAATGGGTGGA-CCTGAGCAGCAA	1.4 kb
232	10804-L20709	Exon 6	2 nt after exon 6	ACTGTCCACGGT-GAGGGGGCCCTG	0.6 kb
244	17124-L20307	Exon 7	868-869	CTATCAGCCTTC-CGCCGCACGTGA	0.4 kb
	No probe	Exon 8			
257	03154-L20711	Exon 9	925-926	CATGAGGACACT-GGGATTCTGGAC	1.2 kb
	No probe	Exon 10			
	No probe	Exon 11			
294 #	03155-L20757	Exon 12	1108-1109	ATGCTAAAGAGG-CTCAAGGGCATG	2.7 kb
309	20995-L32070	Exon 13	1231-1232	GACCATGACGCT-GAGGTCAAATGG	0.4 kb
	No probe	Exon 14			
160	08901-L08992	Exon 15	1308-1309	CATCGGTGCCAA-GCGTACCCTGAC	0.4 kb
	No probe	Exon 16			
148	08902-L11418	Exon 17	1569-1570	CCGGTTCAAGAA-GGACGGGCAGAG	0.6 kb
400	08903-L20707	Exon 18	1719-1720	CGCAGACCTGAT-GGTGGGCGCAAA	0.9 kb
337	03959-L20754	Exon 19	1898-1899	AGGCTGACTACA-GCTTTGTGCCCG	1.5 kb
	No probe	Exon 20			
355	17126-L20309	Exon 21	2050-2051	GTAGCTGGAAAT-AAGCTACGTCTG	0.4 kb
184 ±	03158-L02599	Exon 22	2180-2179, reverse	CACCCACTCATC-GCTGTCACCTGT	0.8 kb
372	08904-L20704	Exon 23	2319-2320	CACAGTGAAGAA-CCCTGTGGGCGA	0.8 kb
328	08905-L11421	Exon 24	2412-2413	CGTGGGAGAGGA-CTCCTGCACAGT	0.2 kb
211	03159-L02600	Exon 25	2541-2542	GATTCAGGAGCT-GAGTCATGAAGC	1.5 kb
409	08906-L20708	Exon 26	2705-2706	ACGTCTCTGACA-CCACGGTCTCCC	0.9 kb
238	03160-L02601	Exon 27	2899-2900	CGGGCACACAAT-ATGGCAGGGCCT	1.1 kb
436	17127-L20310	Exon 28	3009-3010	GACCATTCAGAA-GAAGGTCGGGGA	0.4 kb
265	10805-L11445	Exon 29	3244-3245	CTGCAGGTTGTT-GGTGCGTGGCCA	0.4 kb
274	17128-L20311	Exon 30	3379-3380	GCCGACAAGAAG-ACCATGGTGAGC	0.3 kb
364	08907-L20703	Exon 31	3444-3445	GCCAGAGCTCAT-CATTGGCAATGG	0.2 kb
445	17129-L20312	Exon 32	3572-3573	CACCCAACTATA-AGGCCCTGGACT	0.5 kb
301	03162-L20702	Exon 33	3750-3751	CATGTTCAGCAA-GCAGGGAGTGTT	0.3 kb
227	17130-L20313	Exon 34	3880-3881	GTGCCTCAGTGA-CCAGGCTGGCTC	0.2 kb
322	03163-L20753	Exon 35	3983-3984	TTGGATGTATGT-GTGACAAGTGTG	1.7 kb
		stop codon	*3878-3880 (Exon 34)*		
136 ¬	08908-L08999	*MADD* exon 38		TGGGTTTGCACA-TCTGTCTGCAGG	1.3 kb
280 ¬	20994-L32069	*MADD* exon 37		CGATTGCTCTTA-GTGTTGAACTTT	

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.

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.
± A SNP could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.

Related SALSA MLPA probemixes

P196 TNNT2-BAG3	Familial Hypertrophic Cardiomyopathy, genes included: *TNNT2* and *BAG3*.
P168 ARVC-PKP2	Arrhythmogenic right ventricular cardiomyopathy/dysplasia, genes included: *PKP2*, *DSP*, *JUP*, *DSC2*, *DSG2*, *TGFB3* and *RYR2*

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 P100 MYBPC3

- Bagnall RD et al. (2010). The role of large gene deletions and duplications in MYBPC3 and TNNT2 in patients with hypertrophic cardiomyopathy. *Int J Cardiol.* 145:150-153.
- Chanavat V et al. (2012). Molecular characterization of a large MYBPC3 rearrangement in a cohort of 100 unrelated patients with hypertrophic cardiomyopathy. *Eur J Med Genet.* 55:163-166.
- Kolokotronis K et al. (2019). Biallelic mutation in MYH7 and MYBPC3 leads to severe cardiomyopathy with left ventricular noncompaction phenotype. *Hum Mutat.*
- Nfonsam L et al. (2019). ALU transposition induces familial hypertrophic cardiomyopathy. *Mol Genet Genom Med*, e951.
- Pezzoli L et al. (2012). A new mutational mechanism for hypertrophic cardiomyopathy. *Gene.* 507:165-169.
- Wessels MW et al. (2015). Compound heterozygous or homozygous truncating MYBPC3 mutations cause lethal cardiomyopathy with features of noncompaction and septal defects. *Eur J Hum Genet.* 23:922-928.

P100 Product history	
Version	*Modification*
C2	Five reference probes have been replaced and two probe lengths have been adjusted.
C1	The number of MYBPC3 probes has been increased from 22 to 29. In addition, the 88 and 96 nt control fragments have been replaced (QDX2).
B1	The number of MYBPC3 probes has been increased from 13 to 22. In addition, four extra control fragments at 88-96-100-105 nt have been included.
A1	First release.

Implemented changes in the product description

Version C2-01 — 30 January 2020 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the

amplification products.

Version C1-01 – 04 July 2018 (01P)
- Product description restructured and adapted to a new template.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 15 (55) – 13 September 2016
- Product description adapted to a new lot (lot number changed, small changes in Table 1 and 2, new picture included).

Version 14 (55) – 25 November 2015
- Product description adapted to a new lot (lot number changed, small changes in Table 1 and 2, new picture included).
- New literature references added.

Version 13 (54) – 22 July 2015
- Figure based on the use of old MLPA buffer (replaced in December 2012) removed.
- Various minor textual changes throughout the document.

Version 12 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200