Product Description SALSA® MLPA® Probemix P323-B2 CDK4-HMGA2-MDM2

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, two flanking probes and two new reference probes have been added and two reference probes have been replaced. In addition, multiple probes have a change in length but not in the sequence detected. For complete product history see page 8.

Catalogue numbers:
- **P323-025R:** SALSA MLPA Probemix P323 CDK4-HMGA2-MDM2, 25 reactions.
- **P323-050R:** SALSA MLPA Probemix P323 CDK4-HMGA2-MDM2, 50 reactions.
- **P323-100R:** SALSA MLPA Probemix P323 CDK4-HMGA2-MDM2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. 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 P323 CDK4-HMGA2-MDM2 is a **research use only (RUO)** assay for the detection of copy number alterations of *CDK4*, *HMGA2*, *MDM2* and other genes on chromosome 12.

Alterations of the *CDK4, MDM2* and *HMGA2* genes are suggested to be of diagnostic, clinical and/or prognostic relevance in liposarcoma, osteosarcoma, rhabdomyosarcoma, adenomas and carcinomas of the salivary gland, and in pituitary adenomas. In well-differentiated (WDLPS) and dedifferentiated (DDLPS) types of liposarcomas, the *MDM2* and *HMGA2* genes are recurrently amplified, which can differentiate them from benign lipomas (Italiano et al. 2008). DDLPS and WDLPS patients with only *HMGA2-MDM2* amplification are suggested to have a favourable prognosis compared to patients with both *HMGA2-MDM2* and *CDK4* amplifications (Italiano et al. 2009). In osteosarcoma (OS), *MDM2-CDK4* amplification can be used in differential diagnostics, as it seems to be most prevalent in parosteal OS (Mejia-Guerrero et al. 2010). Amplifications of the 12q13-14 region (including the *GLI1*, *TSPAN31*, *CDK4*, *HMGA2* and *MDM2* genes) are common in leiomyosarcoma and alveolar, embryonal and sclerosing rhabdomyosarcoma, and correlate with poor survival in alveolar rhabdomyosarcoma (Barr et al. 2009). *HMGA2* amplifications are characteristic for pituitary adenomas, and especially for prolactiomas (Finelli et al. 2002) and also observed in adenomas and carcinomas of salivary glands (Persson et al. 2009).

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/

Probemix content: The SALSA MLPA Probemix P323-B2 CDK4-HMGA2-MDM2 contains 50 MLPA probes with amplification products between 124 and 478 nucleotides (nt). This includes 36 probes for detecting copy number changes in chromosome 12, including two probes for the *CDK4* gene at 12q14.1, five probes for the *HMGA2* gene at 12q14.3 (one for each exon) and four probes for the *MDM2* gene at 12q15. In

addition, 14 reference probes are included which target relatively copy number stable regions in various cancer types. Complete probe sequences are 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 or 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls result in only five major peaks shorter than 120 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 120 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-250 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola 2012.

Required specimens: Extracted DNA, which includes DNA derived from paraffin-embedded tissues, 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: 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 healthy individuals without a history of cancer 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 all probes in the reference samples should be <0.10. When this criteria is fulfilled, the following cut-off values for the dosage quotient (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

Please note that these above mentioned dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- 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 (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 are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the major cause of genetic alterations in the *CDK4*, *HMGA2* and *MDM2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P323 CDK4-HMGA2-MDM2.
- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample.

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.

COSMIC mutation database: http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNPs and unusual results (e.g., a duplication of *HMGA2* exons 1 and 3 but not exon 2) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P323-B2 CDK4-HMGA2-MDM2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	12p arm	12q arm	Location (hg18) in kb
64-105	Control fragments – see table in probemix content section for more information				
124 ¥	Reference probe 21547-L02274	18q11			18-019.394
130 ¥	Reference probe 18946-L27359	5q31			05-132.038
136	**MAP3K12 probe** 15901-L17994			12q13.13	12-052.167
142	**CDK4 probe** 03173-L02512			12q14.1	12-056.431
149 ¥	**HMGA2 probe** 15075-L30701			12q14.3	12-064.519
154	**GLI1 probe** 15902-L17995			12q13.3	12-056.145
160	**TBX5 probe** 05694-L05136			12q24.21	12-113.288
166	Reference probe 14281-L15951	15q13			15-025.904
172	**CCND2 probe** 03177-L02516		12p13.32		12-004.253
179 ¥	Reference probe 04446-L30705	4q13			04-068.302
184	**KRAS probe** 10517-L11071		12p12.1		12-025.289
191 ¥	**MDM2 probe** 07182-L30706			12q15	12-067.505
196	Reference probe 05300-L04688	3q11			03-095.088
202	**TSPAN31 probe** 15903-L18385			12q14.1	12-056.426
208	**ALX1 probe** 14414-L16627			12q21.31	12-084.198
217 *	Reference probe 08940-L31205	11p15			11-020.606
226	**PIWIL1 probe** 09841-L18685			12q24.33	12-129.422
232	**FOXM1 probe** 07325-L18686		12p13.33		12-002.848
238	**CHFR probe** 02738-L18389			12q24.33	12-131.974
245 *	**COL2A1 probe** 15452-L30794			12q13.11	12-046.666
250 ¥	Reference probe 07239-L30707	3p11			03-087.396
256 ¥	**HNF1A probe** 07717-L30708			12q24.31	12-119.922
265 ¥	**MDM2 probe** 07183-L30795			12q15	12-067.509
269 ¥	**CDK4 probe** 15904-L30796			12q14.1	12-056.429
275 ¥	**YEATS4 probe** 15905-L30797			12q15	12-068.040
282	**HMGA2 probe** 16186-L16821			12q14.3	12-064.505
288 *	Reference probe 15880-L30312	2p16			02-050.317
296 ¥	Reference probe 07017-L30703	14q11			14-020.826
301 ¥	**RAN probe** 15906-L30798			12q24.33	12-129.923
310	**IGF1 probe** 02340-L01834			12q23.2	12-101.394
317	**CDKN1B probe** 16517-L18978		12p13.1		12-012.762
324	**MIR26A2 probe** 16903-L20362			12q14.1	12-056.505
331	**DDIT3 probe** 15907-L20363			12q13.3	12-056.197
339	**MDM2 probe** 02894-L20364			12q15	12-067.488
346	**GLI1 probe** 15908-L18001			12q13.3	12-056.150
355 ¥	**RAN probe** 21745-L30799			12q24.33	12-129.925
362	**KIF21A probe** 05762-L18394			12q12	12-037.975
370	**MDM2 probe** 00337-L18786			12q15	12-067.504
378	Reference probe 06216-L20365	16p11			16-031.393
385	Reference probe 05914-L05359	18p11			18-013.724
392	**CCND2 probe** 03178-L18979		12p13.32		12-004.283
400	**CDK2 probe** 14405-L16087			12q13.2	12-054.647
409	**PTPN11 probe** 12523-L13573			12q24.13	12-111.341
418	**HMGA2 probe** 15074-L16832			12q14.3	12-064.508
426 ¥	**HMGA2 probe** 21744-L16847			12q14.3	12-064.643
436 *	Reference probe 15731-L30702	21q11			21-014.668
445	**HMGA2 probe** 15086-L16849			12q14.3	12-064.595
456	Reference probe 13470-L20366	2q13			02-113.719
469 *	**STAT6 probe** 21911-L30704			12q13.3	12-055.788
478 *	Reference probe 21578-L30146	4q22			04-089.208

* New in version B2 (from lot B2-0718 onwards).

¥ Changed in version B2 (from lot B2-0718 onwards). Small change in length, no change in sequence detected.

Table 2. P323-B2 probes arranged according to chromosomal location

Table 2a. Chromosome 12

Length (nt)	SALSA MLPA probe	Gene / Exon	Location/ Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
12p chromosomal arm					
232	07325-L18686	*FOXM1*	12p13.33	CCATGATACAAT-TCGCCATCAACA	1.4 Mb
172	03177-L02516	*CCND2*	12p13.32	AGACCAGTTTTA-AGGGGAGGACCG	29.9 **k**b
392	03178-L18979	*CCND2*	12p13.32	TAACAGCCAAGA-AGCCTGCAGGAG	8.5 Mb
317	16517-L18978	*CDKN1B*	12p13.1	CGCGCTCCTAGA-GCTCGGGCCGTG	12.5 Mb
184	10517-L11071	*KRAS*	12p12.1	ATTTTGTGGACG-AATATGATCCAA	12.7 Mb
12q chromosomal arm					
362	05762-L18394	*KIF21A*	12q12	AGGCTCGCAATT-TGCAAGATGGTC	8.7 Mb
245	15452-L30794	*COL2A1*	12q13.11	TGTGTACCCTTG-TAGGGAGCCCCT	5.5 Mb
136	15901-L17994	*MAP3K12*	12q13.13	GCATCCAGAGTT-CGAGCTGACGAG	2.5 Mb
400	14405-L16087	*CDK2*	12q13.2	CATTGTTTCAAG-TTGGCCAAATTG	1.1 Mb
469	21911-L30704	*STAT6*	12q13.3	CCGACGCCTTCT-GCTGCAACTTGG	0.4 Mb
154	15902-L17995	*GLI1*	12q13.3	ACTCGCGATGCA-CATCTCCAGGAG	4.8 **k**b
346	15908-L18001	*GLI1*	12q13.3	GGACCAGCTACA-TCAACTCCGGCC	47.1 **k**b
331	15907-L20363	*DDIT3*	12q13.3	CCTCTCACTAGT-GCCAATGATGTG	0.2 Mb
202	15903-L18385	*TSPAN31*	12q14.1	TCCACATCATCG-GCGGAGTCATTG	2.8 **k**b
269	15904-L30796	*CDK4*, ex 8	NM_000075.2; 1062-1063	TGCTGACTTTTA-ACCCACACAAGC	2.7 **k**b
142	03173-L02512	*CDK4*, ex 3	NM_000075.2; 505-506	AACCCTGGTGTT-TGAGCATGTAGA	73.4 **k**b
324	16903-L20362	*MIR26A2*	12q14.1	AGGCCTCACAGA-TGGAAACAGCCT	8.0 Mb
282	16186-L16821	*HMGA2*, ex 1	NM_003483.4; 357-358	CCGCCTAACATT-TCAAGGGACACA	3.2 **k**b
418	15074-L16832	*HMGA2,* ex 2	NM_003483.4; 962-963	GACCCAGGGGAA-GACCCAAAGGCA	10.5 **k**b
149	15075-L30701	*HMGA2,* ex 3	NM_003483.4; 1029-1030	AGCCACTGGAGA-AAAACGGCCAAG	76.8 **k**b
445+	15086-L16849	*HMGA2,* int 3 (ex 4)	NM_003483.4; 36.1 kb before exon 4	CCAAGATGTAGT-TTCACTGCTACC	48.0 **k**b
426	21744-L16847	*HMGA2*, ex 5	NM_003483.4; 1217-1218	AGTGACCACTTA-TTCTGTATTGCC	2.8 Mb
339	02894-L20364	*MDM2,* ex 1	NM_002392.5; 133-134	CGAGATCCTGCT-GCTTTCGCAGCC	16.1 **k**b
370	00337-L18786	*MDM2,* ex 6	NM_002392.5: 691-692	GTACATCTGTGA-GTGAGAACAGGT	0.2 **k**b
191	07182-L30706	*MDM2,* ex 7	NM_002392.5; 768-769	GAGAAACCTTCA-TCTTCACATTTG	4.2 **k**b
265	07183-L30795	*MDM2,* ex 8	NM_002392.5; 877-878	GAAAACGCCACA-AATCTGATAGTA	0.5 Mb
275	15905-L30797	*YEATS4*	12q15	TATGTTCAAGAG-AATGGCCGAATT	16.2 Mb
208	14414-L16627	*ALX1*	12q21.31	GTCTGCAGGCAA-ATGCGTGCAGGC	17.2 Mb
310	02340-L01834	*IGF1*	12q23.2	AGGTAGAAGAGA-TGCGAGGAGGAC	9.9 Mb
409	12523-L13573	*PTPN11*	12q24.13	CAGGAGGAAGCA-AGGATGCTTTGG	1.9 Mb
160	05694-L05136	*TBX5*	12q24.21	GTGAGGCAAAAA-GTGGCCTCCAAC	6.6 Mb
256	07717-L30708	*HNF1A*	12q24.31	GCCTCAGTGTCT-GAGGTGAAGACC	9.5 Mb
226	09841-L18685	*PIWIL1*	12q24.33	CAGAGAGCCAAA-TCTGTCACTGTC	0.5 Mb
301	15906-L30798	*RAN*	12q24.33	GTGTTTTTCAAC-AGCTTGTATTGG	1.7 **k**b
355	21745-L30799	*RAN*	12q24.33	GTACTAATTCCC-ACAAATGTTTCT	2.0 Mb
238	02738-L18389	*CHFR*	12q24.33	GGCGGCGGCGCT-CACCAAGAGCGG	-

+ This probe has a ligation site between positions 1322-1323 in the transcript variant in RefSeq NM_003484.1.

Note: The *HMGA2* exon numbering has changed. From description version B2-01 onwards, we have adopted the NCBI exon numbering that is present in the NM_003483.4 sequence for this gene. The exon numbering used in this P323-B2 CDK4-HMGA2-MDM2 product description for *CDK4* is the exon numbering from the RefSeq transcript NM_000075.2, which is identical to the LRG_490 sequence. For *MDM2*, exon numbering is from the RefSeq transcript NM_002392.5. The exon numbering and NM sequences used are from 07/2018 but can be changed by NCBI after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2b. Reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
288	15880-L30312	*NRXN1*	2p16	GAGTGGACAGTT-CTTCAGGCTTGG	63.4 Mb
456	13470-L20366	*PAX8*	2q13	TTGCAGATGCTA-GGACACAAGAGA	-
250	07239-L30707	*POU1F1*	3p11	TCCTATACACCA-GCCTCTTCTGGC	7.7 Mb
196	05300-L04688	*PROS1*	3q11	CATTTAAATCCC-CAGCATAAATCA	-
179	04446-L30705	*GNRHR*	4q13	TGGAACATTACA-GTCCAATGGTAT	20.9 Mb
478	21578-L30146	*PKD2*	4q22	CCCTCCTTCTGG-AGCTATGTCCGC	-
130	18946-L27359	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	-
217	08940-L31205	*SLC6A5*	11p15	TTGCCTCTCAGG-TGTGGAAAGATG	-
296	07017-L30703	*RPGRIP1*	14q11	CTACATCAGGAG-ACTTGCCAGTTA	-
166	14281-L15951	*OCA2*	15q13	GCCGCGATGAGA-CAGAGCATGATG	-
378	06216-L20365	*TGFB1I1*	16p11	CAGGAACTTAAT-GCCACTCAGTTC	-
385	05914-L05359	*RNMT*	18p11	TACAATGAACTT-CAGGAAGTTGGT	5.7 kb
124	21547-L02274	*NPC1*	18q11	GACGAGTCTGTG-GATGAGGTCACA	-
436	15731-L30702	*HSPA13*	21q11	GACCTAGCAGTA-GTAACGGGAGTG	-

Related SALSA MLPA probemixes

- P419 CDKN2A/2B-CDK4: Contains more probes for the *CDK4* gene.
- P175 Tumour Gain: Contains two other probes for the *MDM2* gene.

References

- Barr F et al. (2009). Genomic and clinical analyses of 2p24 and 12q13-q14 amplification in alveolar rhabdomyosarcoma: a report from the Children's Oncology Group. *Genes Chromosomes Cancer*. 48:661-72.
- Finelli P et al. (2002). The High Mobility Group A2 gene is amplified and overexpressed in human prolactinomas. *Cancer Res*. 62:2398-405.
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- Italiano A et al. (2008). HMGA2 is the partner of MDM2 in well-differentiated and dedifferentiated liposarcomas whereas CDK4 belongs to a distinct inconsistent amplicon. *Int J Cancer*. 122:2233-41.
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- 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 P323 CDK4-HMGA2-MDM2

- Fusco I et al. (2016). Variations in the high-mobility group-A2 gene (HMGA2) are associated with idiopathic short stature. *Pediatr Res*. 79:258-61.
- Heldt F et al. (2018). 12q14 microdeletion syndrome: A family with short stature and Silver-Russell syndrome (SRS)-like phenotype and review of the literature. *Eur J Med Genet.* 61:421-7.

P323 Product history	
Version	*Modification*
B2	Two flanking probes and two new reference probes have been added and two reference probes have been replaced. In addition, multiple probes have a change in length but not in the sequence detected.
B1	Probemix has been completely redesigned. Probes for HMGA2 and several other genes at 12p and 12q have been included. In addition, the 88 and 96 nt control fragments have been replaced (QDX2).
A1	First release.

Implemented changes in the product description

Version B2-01 – 25 July 2018 (01P)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- All warnings have been removed.
- Various minor textual and layout changes.

Version 07 – 11 December 2015 (T08)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Reference added on page 2.
- Warning added in Table 1 and 2, 160 nt probe 05694-L05136.
- Warning added in Table 1 and 2 about reference probe at 436 nt (05504-L04927).
- Various minor layout changes.
- Mapview locations removed from Table 2 and added to Table 1.
- Ligation sites and transcript numbers indicated for several probes in Table 2.

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

Version 05 (48)
- Warning added in Table 1, 142 nt probe 03173-L02512 and 418 nt probe 15074-L16832.

Version 04 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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