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

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 11.

Catalogue numbers:
- **P323-025R:** SALSA MLPA Probemix P323-B2 CDK4-HMGA2-MDM2, 25 reactions.
- **P323-050R:** SALSA MLPA Probemix P323-B2 CDK4-HMGA2-MDM2, 50 reactions.
- **P323-100R:** SALSA MLPA Probemix P323-B2 CDK4-HMGA2-MDM2, 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 P323 CDK4-HMGA2-MDM2 is a **research use only (RUO)** assay for the detection of deletions, gains or amplifications 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-q14 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 prolactinomas (Finelli et al. 2002), and are also observed in adenomas and carcinomas of salivary glands (Persson et al. 2009).

In addition, the P323 CDK4-HMGA2-MDM2 probemix can be used for the analysis of other tumour types to detect copy number alterations affecting genes on chromosome 12, that are targeted by this P323 probemix. These include 12q chromosomal arm copy number alterations resulting in *CDK4* and *MDM2* amplification in glioma's (Reifenberger et al. 1993; Reifenberger et al., 1996; Rollbrocker at al. 1996; Hoadly et al. 2018), *CCND2* amplifications in colon adenocarcinoma, ovarian serous adenocarcinoma and testicular germ cell tumours (AACR Project GENIE Consortium, 2017; Hoadly et al. 2018), copy number loss within the 12p chromosomal arm in multiple myeloma (Munshi et al. 2011; Hung et al. 2021) and acute lymphoblastic leukemia (ALL) (Raynaud et al. 1996; Wiemels et al. 2008), and trisomy 12 observed in chronic lymphocytic leukemia (CLL) (Döhner et al. 2000; Haferlach et al. 2007; Autore et al. 2018).

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/

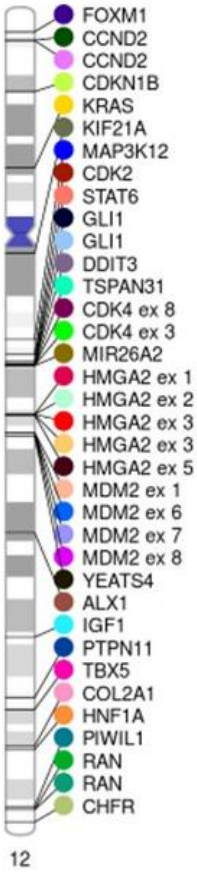

Figure 1: Phenogram plot showing a schematic overview of chromosome 12-specific probes in the P323 probemix (http://visualization.ritchielab.org/phenograms/plot)

Exon numbering
The exon numbering used in this P323-B2 CDK4-HMGA2-MDM2 product description is the exon numbering from the LRG or RefSeq sequence, as indicated in Table 2. For *CDK4*, LRG_490 is available at www.lrg-sequence.org. For *HMGA2* and *MDM2*, the exon numbering is from the RefSeq transcripts NM_003483.6 and NM_002392.6, respectively. 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 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 *CDK4* at 12q14.1, five probes for *HMGA2* at 12q14.3 (one for each exon) and four probes for *MDM2* at 12q15. In addition, 14 reference probes are included which target relatively copy number stable regions in various cancer types. Partial 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)

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.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 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 (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 samples described in the table below have been tested with this P323-B2 probemix at MRC Holland, and can be used as positive control samples to detect copy number alterations. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA (hg18)*	Altered genes in P323-B2	Expected copy number alteration
HCC-827◊	DSMZ	12q14.1	*CDK4* and *TSPAN31*	Amplification
		12q14.3	*HMGA2* exon 1-3	Amplification
HCC-1143◊	DSMZ	12q13.3–q14.1	*STAT6, GLI1, DDIT3, TSPAN31, CDK4* and *MIR26A2*	Gain
		12q14.3	*HMGA2*	Amplification
		12q15	*MDM2* and *YEATS4*	Amplification
DK-MG◊	DSMZ	12q15	*MDM2*	Amplification
IGR-37◊	DSMZ	12q12–q24.33	*KIF21A, COL2A1, MAP3K12, CDK2, STAT6, GLI1, DDIT3, TSPAN31, CDK4, MIR26A2, HMGA2, MDM2, YEATS4, ALX1, IGF1, PTPN11, TBX5, HNF1A, PIWIL1, RAN* and *CHFR*	Loss
COLO-824◊	DSMZ	12p13.32-p13.33	*FOXM1, CCND2* exon 1	Gain
		12p12.1	*CDKN1B, KRAS, KIF21A*	Gain
		12q13.11–q14.1	*COL2A1, MAP3K12, CDK2, STAT6, GLI1, DDIT3, TSPAN31,* and *CDK4*	Loss
		12q24.33	*CHFR*	Loss
NA07981	Coriell	12q13.33–p12.1	*FOXM1, CCND2, CDKN1B* and *KRAS*	Mosaic homozygous duplication
NA08035	Coriell	12q13.33–p12.1	*FOXM1, CCND2, CDKN1B* and *KRAS*	Heterozygous duplication
NA02819	Coriell	12q24.33	*PIWIL1, RAN* and *CHFR*	Heterozygous deletion

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of copy number alteration (CNA) present in this cell line cannot be determined by this P323-B2-CDK4-HMGA2-MDM2 probemix.

◊ In this indicated cell line sample some of the reference / flanking probes are also affected by CNAs.

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. When this criterion is fulfilled, the following cut-off values for the final ratio (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/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios 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 subclonal cases.
- 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.

P323-specific note

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood-derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- Most genetic alterations in cancer are small (point) mutations. If present, these type of mutations in the *CDK4*, *HMGA2* and *MDM2* genes or other genes on chromosome 12, will not be detected by using SALSA MLPA Probemix P323.
- 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.

- 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, especially in solid tumours with more chaotic karyotypes.

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

We strongly encourage users to deposit positive results in the http://cancer.sanger.ac.uk/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 SNVs and unusual results (e.g., a duplication of *HMGA2* exons 1 and 3, but not exon 2) to MRC Holland: info@mrcholland.com.

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

Length (nt)	SALSA MLPA probe	Chromosomal location (hg18)			Location in hg18 (in kb)
		Reference	12p arm	12q arm	
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
394	**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

± SNV rs375955080 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

Table 2a. P323 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location / ligation site	Partial sequence (24nt 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 **kb**
394	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	357,1 **kb**
154	15902-L17995	*GLI1*	12q13.3	ACTCGCGATGCA-CATCTCCAGGAG	4,8 **kb**
346	15908-L18001	*GLI1*	12q13.3	GGACCAGCTACA-TCAACTCCGGCC	47,1 **kb**
331	15907-L20363	*DDIT3*	12q13.3	CCTCTCACTAGT-GCCAATGATGTG	229,1 **kb**
202	15903-L18385	*TSPAN31*	12q14.1	TCCACATCATCG-GCGGAGTCATTG	2,8 **kb**
269	15904-L30796	*CDK4*, ex 8	NM_000075.4; 990-991	TGCTGACTTTTA-ACCCACACAAGC	2,7 **kb**
142	03173-L02512	*CDK4*, ex 3	NM_000075.4; 433-434	AACCCTGGTGTT-TGAGCATGTAGA	73,4 **kb**
324	16903-L20362	*MIR26A2*	12q14.1	AGGCCTCACAGA-TGGAAACAGCCT	8 Mb
282	16186-L16821	*HMGA2*, ex 1	NM_003483.6; 334-335	CCGCCTAACATT-TCAAGGGACACA	3,2 **kb**
418	15074-L16832	*HMGA2*, ex 2	NM_003483.6; 939-940	GACCCAGGGGAA-GACCCAAAGGCA	10,5 **kb**
149	15075-L30701	*HMGA2*, ex 3	NM_003483.6; 1006-1007	AGCCACTGGAGA-AAAACGGCCAAG	76,8 **kb**
445	15086-L16849	*HMGA2*, int 3	NM_003483.6; 36.1 kb before exon 4; NM_003484.1; 1322-1323	CCAAGATGTAGT-TTCACTGCTACC	48,0 **kb**
426	21744-L16847	*HMGA2*, ex 5	NM_003483.6; 1194-1195	AGTGACCACTTA-TTCTGTATTGCC	2.8 Mb
339	02894-L20364	*MDM2*, ex 1	NM_002392.6; 128-129	CGAGATCCTGCT-GCTTTCGCAGCC	16,1 **kb**
370	00337-L18786	*MDM2*, ex 6	NM_002392.6; 686-687	GTACATCTGTGA-GTGAGAACAGGT	0,2 **kb**
191	07182-L30706	*MDM2*, ex 7	NM_002392.6; 763-764	GAGAAACCTTCA-TCTTCACATTTG	4,2 **kb**
265	07183-L30795	*MDM2*, ex 8	NM_002392.6; 872-873	GAAAACGCCACA-AATCTGATAGTA	531,2 **kb**
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	501,3 **kb**
301	15906-L30798	*RAN*	12q24.33	GTGTTTTTCAAC-AGCTTGTATTGG	1,7 **kb**
355	21745-L30799	*RAN*	12q24.33	GTACTAATTCCC-ACAAATGTTTCT	2 Mb
238	02738-L18389	*CHFR*	12q24.33	GGCGGCGGCGCT-CACCAAGAGCGG	-

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

± SNV rs375955080 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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.

Table 2b. Reference probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene	Location	Partial sequence (24nt adjacent to ligation site)	Location in hg18 (in kb)
288	15880-L30312	*NRXN1*	2p16	GAGTGGACAGTT-CTTCAGGCTTGG	02-050,317
456	13470-L20366	*PAX8*	2q13	TTGCAGATGCTA-GGACACAAGAGA	02-113,719
250	07239-L30707	*POU1F1*	3p11	TCCTATACACCA-GCCTCTTCTGGC	03-087,396
196	05300-L04688	*PROS1*	3q11	CATTTAAATCCC-CAGCATAAATCA	03-095,088
179	04446-L30705	*GNRHR*	4q13	TGGAACATTACA-GTCCAATGGTAT	04-068,302
478	21578-L30146	*PKD2*	4q22	CCCTCCTTCTGG-AGCTATGTCCGC	04-089,208
130	18946-L27359	*IL4*	5q31	ATCGACACCTAT-TAATGGGTCTCA	18-013,724
217	08940-L31205	*SLC6A5*	11p15	TTGCCTCTCAGG-TGTGGAAAGATG	11-020,606
296	07017-L30703	*RPGRIP1*	14q11	CTACATCAGGAG-ACTTGCCAGTTA	14-020,826
166	14281-L15951	*OCA2*	15q13	GCCGCGATGAGA-CAGAGCATGATG	15-025,904
378	06216-L20365	*TGFB1I1*	16p11	CAGGAACTTAAT-GCCACTCAGTTC	16-031,393
385	05914-L05359	*RNMT*	18p11	TACAATGAACTT-CAGGAAGTTGGT	18-013,724
124	21547-L02274	*NPC1*	18q11	GACGAGTCTGTG-GATGAGGTCACA	18-019,394
436	15731-L30702	*HSPA13*	21q11	GACCTAGCAGTA-GTAACGGGAGTG	21-014,668

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.
- **P425 Multiple Myeloma**: Contains probes for chromosomal arm 12p.
- **P105 Glioma**: Contains one other *CDK4* probe and two other probes for *MDM2*.
- **P040 CLL**: Contains several other probes for chromosome 12.
- **P335 ALL-IKZF1**: Contains several other probes for chromosome 12.

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Selected publications using SALSA MLPA Probemix P323-B2-CDK4-HMGA2-MDM2

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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 B2-04 – 02 June 2025 (04P) - Adjusted information regarding complete probe sequences being available on the website. - Various minor textual changes. Version B2-03 – 31 January 2023 (04P) - Information in the Positive control DNA samples table on page 4 has been updated for sample NA07981: mosaic homozygous duplication instead of heterozygous duplication. Version B2-02- 20 July 2022 (04P) - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2a). - General information updated: relevance of the P323-B2 CDK4-HMGA2-MDM2 probemix for the detection of copy number alterations in chr. 12 for general tumour characterization is described. - Table added with positive control DNA samples on page 4. - Warning added in Table 1 and 2a, 160 nt probe 05694-L05136. - Phenogram plot added (Figure 1), showing a schematic overview of chromosome 12-specific probes in the P323 probemix. - Various minor textual and layout changes.

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