Product Description SALSA® MLPA® Probemix P056-D1 TP53

To be used with the MLPA General Protocol.

Version D1. Two flanking probes of TP53 and all flanking probes of CHEK2 have been removed. One CHEK2 probe has been added and one CHEK2 probe has been replaced. Moreover, nine probes have been changed in length but not in the sequence detected. For complete product history see page 10.

Catalogue numbers:
- **P056-025R:** SALSA MLPA probemix P056 TP53, 25 reactions.
- **P056-050R:** SALSA MLPA probemix P056 TP53, 50 reactions.
- **P056-100R:** SALSA MLPA probemix P056 TP53, 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 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.

Intended use: The SALSA MLPA probemix P056 TP53 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *TP53* gene in order to confirm a potential cause and clinical diagnosis for Li-Fraumeni syndrome (LFS1) or Li-Fraumeni-like syndrome (LFL). In addition, this assay can be used to detect deletions or duplications in the human *CHEK2* gene exons 8, 10 and 13 and the *CHEK2* 1100delC mutation, to determine a suggested cause for Li-Fraumeni syndrome (LFS2) in a research setting. This assay can also be used for molecular genetic testing of at-risk family members.

This assay is for use with human DNA extracted from peripheral blood. In a research setting this assay can be used on DNA derived from fresh or formalin-fixed paraffin-embedded tumour tissue. Most defects in the *TP53* gene are point mutations, the majority of which are not detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of this gene. Deletions or duplications detected with the P056 TP53 probemix should be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. This probemix is not intended to be used as a standalone assay for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Li-Fraumeni syndrome (LFS) is a clinically and genetically heterogeneous inherited cancer syndrome. LFS1 is characterised by autosomal dominant inheritance and early onset of tumours, multiple tumours within an individual, and multiple affected family members. The most common types of tumours are soft tissue sarcomas and osteosarcomas, pre-menopausal breast cancer, brain tumours, leukaemia, and adrenocortical carcinoma. Approximately 70% of LFS1 cases contain germline mutations in the tumour suppressor gene *TP53*. Families presenting incomplete features of LFS are referred as having Li-Fraumeni-like syndrome (LFL) and around 20-40% of these patients have a germline mutation in *TP53* (Ruijs et al. 2010). Somatic pathogenic variants in *TP53* are found in about 50% of all tumours, making it one of the most frequently altered genes in human cancers. Around 50% of the individuals carrying germline mutations in *TP53* will develop cancer by the age of 30 years with a lifetime risk of up to 70% in men and almost 100% in women. There is no definitive information on the optimal methods and efficacy of tumour

surveillance for children or adults with a germline *TP53* pathogenic variant. For more information: https://www.ncbi.nlm.nih.gov/books/NBK1311/.

A second form of Li-Fraumeni syndrome (LFS2) is caused by mutations in the *CHEK2* gene. In particular, the *CHEK2* 1100delC mutation has been suggested as an underlying cause, though also a deletion in *CHEK2* was found in a patient fulfilling LFL criteria (Ruijs et al. 2009). Researchers are uncertain whether *CHEK2* gene mutations actually cause LFS or are merely associated with an increased risk of several types of cancer, including those cancers often seen in LFS. For more information: https://omim.org/entry/609265.

Gene structure:
The *TP53* gene spans ~19 kilobases (kb) on chromosome 17p13.1, about 7.5 Mb from the p-telomere. The exon numbering is according to LRG_321 (www.lrg-sequence.org) and is identical to GenBank NG_017013.2. The *CHEK2* gene is located on chromosome 22q12.1 and spans ~55 kb. The preliminary *CHEK2* LRG_302 is still pending final approval (www.lrg-sequence.org) and is identical to GenBank NG_008150.1.

Transcript variants: *TP53* has multiple transcript variants of which transcript variant 1 (NM_000546.5; 2591 nt; coding sequence 203-1384; https://www.ncbi.nlm.nih.gov/gene/7157) encodes isoform a (also known as p53alpha), which is the longest isoform. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 2 and the stop codon in exon 11. Transcript variant 3 (NM_001126114.2) encodes isoform b (also known as p53beta) and has an additional exon in the 3' coding region, resulting in a shorter and distinct C-terminus compared to isoform a.
For *CHEK2,* transcript variant 1 is the most abundant and encodes isoform a (NM_007194.4; 1844 nt; coding sequence 59-1690; https://www.ncbi.nlm.nih.gov/gene/11200). This sequence is a reference standard in the RefSeqGene project.

Exon numbering: The *TP53* exon numbering used in this P056-D1 TP53 product description corresponds to exon numbering of LRG_321 transcript t1 (NM_000546.5), and transcript t3 (NM_001126114.2). For *CHEK2*, the exon numbering of LRG_302 is used. The exon numbering and NM sequences used are from 09/2018, but can be changed by NCBI after the release of the product description.

Probemix content: This SALSA MLPA probemix P056 TP53 contains 40 probes with amplification products between 129 and 490 nt (Table 1), including 14 reference probes. At least one probe is present for each exon of transcript variant 1 of *TP53*; two probes are present for exon 1 and exon 4b. One probe is included for exon 9a, which is present in transcript variant 3. Several flanking probes for *TP53* are present to determine the extent of the deletion/duplication. In addition, there are four probes included for *CHEK2*: a probe for exon 8, 10 and 13 and a mutation specific probe for the *CHEK2* 1100delC mutation, which will only generate a signal when the mutation is present. The identity of the genes detected by the reference probes is available online (www.mlpa.com) and in Table 2c.

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 or 96 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). For more information on the use of MLPA in tumour applications see 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 from human peripheral blood or, in a research setting, DNA derived from fresh or formalin-fixed paraffin-embedded tumour tissue, 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 unrelated individuals who are from families without a history of LFS or LFL. 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 the 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. Sample ID numbers NA07106 and HG00187 (1000 genome sample) from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control sample to detect a duplication of the *CHEK2* copy number probes and the *CHEK2* 1100delC mutation-specific probe, respectively.

An example on the use of cancer cell line as a positive DNA sample for tumour applications is cell line SK-N-MC (ACC-203) from DSMZ. This cell line has a deletion of one copy of the whole *TP53* gene, which extends to the flanking probes on the 17p arm. In addition, two copies are deleted for *TP53* exons 2a and 2d, indicating a homozygous deletion of exon 2. As this is a tumour cell line, reference probes are more prone to show deviating copy number results, which in this case resulted in 3 copies for reference probe *RAB7A* (16316-L21434) and one copy for reference probe *UPF2* (00979-L21316). As a result, turning the slope correction off in Coffalyser.Net analysis can help to get the correct interpretation when regions targeted by reference probes are affected by their copy number. The quality of cell lines can change, therefore samples should be validated before use.

SALSA Binning DNA SD067: The SD067 Binning DNA provided with this probemix can be used as Binning DNA sample for binning of the *CHEK2* 1100delC mutation-specific probe (208 nt probe, 18318-L26751). SD067 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD067 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s), as for this purpose true mutation positive patient samples or cell lines should be used. It is strongly advised to use DNA sample and reference DNA samples extracted with the same method and derived from the same source of tissue. For further details, please consult the SD067 Binning DNA product description provided online at www.mlpa.com.

Performance characteristics: The frequency of deletions or duplications in *TP53* in LFS1 or LFL is ~1% (https://www.ncbi.nlm.nih.gov/books/NBK1311/, Mouchawar et al. 2010, Schulz et al. 2012) in *TP53* point mutation negative patients. Deletions or duplications in *CHEK2* are rare, whereas the overall prevalence of the *CHEK2* 1100delC mutation in breast cancer is ~0.9%, depending on ethnicity (Zhang et al. 2008).

The analytical sensitivity and specificity for the detection of deletions or duplications in these genes is very high and can be considered >99% (based on a literature review using articles from 2009-2017). Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much

sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must 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 expected results for *TP53* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 0 (homozygous deletion) or 4 (homozygous duplication).

The standard deviation of all probes in the reference samples should be <0.10 and when studying germline mutations the dosage quotient (DQ) of the reference probes 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 or pseudo-autosomal chromosomes:

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 only valid for germline testing. 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 in some cases not result in inactivation of that gene copy.
- Copy number changes detected by only a reference or flanking probe are unlikely to have any relation to the condition tested for.

Notes for P056

- **CHEK2 1100delC probe**: We have received reports of experiments in which a peak for the CHEK2 1100delC probe appeared in *all* samples, which was caused by incomplete ligase inactivation. For more information on this issue, please contact info@mlpa.com. Please note, that this probe will also generate

a signal in the unlikely situation that the mutation is present in the *CHEK2* pseudogene. Results obtained with this CHEK2 mutation probe should therefore be treated with caution.
- The ligation sites of the *TP53* probes 02376-L30912 (exon 4b) and 01999-L21411 (exon 7) are located in a germline and somatic mutational hotspot (http://p53.iarc.fr/). In case of an apparent deletion, the sequence of this region should be sequenced.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in the *TP53* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P056 TP53.
- 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 incorrect 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.
- When used on tumour DNA (for research use only): 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. SALSA MLPA probemix P190 CHEK2 is available for deletion or duplication analysis of other *CHEK2* exons.

Copy number changes detected by one or 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.

TP53 mutation database: http://p53.iarc.fr/ or http://grenada.lumc.nl/LSDB_list/lsdbs/TP53. We strongly encourage users to deposit positive results in the TP53 database. For *CHEK2* the http://grenada.lumc.nl/LSDB_list/lsdbs/chek2 can be used. 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 *TP53* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P056-D1 TP53

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a]		
		Reference	*TP53*	*CHEK2*
64-105	Control fragments – see table in probemix content section for more information			
129	Reference probe 19616-L26684	4p13		
135	Reference probe 16316-L21434	3q21		
140 ¬	**EFNB3 probe** 03962-L21069		17p13.1	
147 ¬	**MPDU1 probe** 19643-L26685		17p13.1	
154 * »	**CHEK2 probe** 21913-L06190			**Exon 10**
160	Reference probe 09787-L10202	15q21		
166	**TP53 probe** 01588-L06028		**Exon 1**	
175	**TP53 probe** 19637-L26296		**Downstream**	
181 * » «	**CHEK2 probe** 21654-L30911			**Exon 13**
187 ¥ ¬	**POLR2A probe** 19647-L30910		17p13.1	
193	Reference probe 11556-L26606	5q31		
199	**TP53 probe** 01996-L26321		**Exon 2a**	
208 » §	**CHEK2 probe** 18318-L26751			**1100delC**
216	**TP53 probe** 02375-L26750		**Exon 2d**	
224 +	**TP53 probe** 19638-L26297		**Exon 9a**	
230	Reference probe 17130-L26574	11p11		
238 ¬	**AKAP10 probe** 19648-L00940		17p11.2	
247	Reference probe 08728-L08739	9q21		
256 ¥ ‡	**TP53 probe** 02376-L30912		**Exon 4b**	
274 ¥	Reference probe 17450-L30913	16p13		
286 ¥ ‡	**TP53 probe** 01999-L21411		**Exon 7**	
299	**TP53 probe** 17420-L21142		**Exon 3**	
310	Reference probe 07028-L06639	14q11		
318	**TP53 probe** 17421-L21315		**Exon 5**	
328	Reference probe 13397-L26608	6q12		
335 ¬	**ATP1B2 probe** 19884-L26749		17p13.1	
346	**TP53 probe** 17422-L21144		**Exon 10**	
359 ¥	**TP53 probe** 22010-L21147		**Exon 4b**	
372	Reference probe 14835-L26609	1p34		
382 ¬	**ATP1B2 probe** 19645-L26316		17p13.1	
391	**TP53 probe** 17423-L21145		**Exon 8**	
401	**TP53 probe** 19650-L21141		**Exon 6**	
409	**TP53 probe** 02263-L01749		**Exon 1**	
420	Reference probe 08839-L08899	2p13		
432 ¥ »	**CHEK2 probe** 06631-L30915			**Exon 8**
447 ¥	**TP53 probe** 17424-L30914		**Exon 11**	
459 ¥ ¬	**POLR2A probe** 09951-L30916		17p13.1	
471	Reference probe 00979-L21316	10p14		
480 ¥	Reference probe 21882-L15817	2q13		
490	Reference probe 19137-L25693	21q22		

(a) The *TP53* exon numbering used in this P056-D1 TP53 product description corresponds to exon numbering of LRG_321 transcript t1 (NM_000546.5), and transcript t3 (NM_001126114.2). For *CHEK2*, the exon numbering of LRG_302 is used. The exon numbering and NM sequences used are from 09/2018, but can be changed by NCBI after the release of the product description.

* New in version D1 (from lot D1-0418 onwards).
¥ Changed in version D1 (from lot D1-0418 onwards). Small change in length, no change in sequence detected.
§ Mutation-specific probe. This probe will only generate a signal when the *CHEK2* 1100delC mutation is present. Results obtained with this *CHEK2* 1100delC probe should be treated with caution! Please read the note on page 4.
¬ 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.

» Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P190.
+ Exon numbering for exon 9a is indicated according to LRG_321 transcript t3 (NM_001126114.2). Exon 9a is present in some alternative transcript variants only.
« 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.
‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

Table 2. P056 probes arranged according to chromosomal location

Table 2a. *TP53*

Length (nt)	SALSA MLPA probe	*TP53* exon[(a)]	Ligation site NM_000546.5	Partial sequence[(b)] (24 nt adjacent to ligation site)	Distance to next probe
238 ¬	19648-L00940	*AKAP10* gene	17p11.2	AGGACCAAGTCA-TGTTGCAATCAA	12254.7 kb
140 ¬	03962-L21069	*EFNB3* gene	17p13.1	TCTCCTAATTAT-GAGTTCTACAAG	20.5 kb
		start codon	*203-205 (exon 2a)*		
409	02263-L01749	Exon 1	67 nt before exon 1	CTTCCTCCGGCA-GGCGGATTACTT	0.2 kb
166	01588-L06028	Exon 1	118-119	TCCGGGGACACT-TTGCGTTCGGGC	10.8 kb
199	01996-L26321	Exon 2a	177-178	CTCTTGCAGCAG-CCAGACTGCCTT	0.2 kb
216	02375-L26750	Exon 2d	290-291	TTCCTGAAAACA-ACGTTCTGGTAA	0.3 kb
299	17420-L21142	Exon 3	511-510 reverse	TAGCTGCCCTGG-TAGGTTTTCTGG	0.8 kb
256 ‡	02376-L30912	Exon 4b	606-607	CAAGATGTTTTG-CCAACTGGCCAA	0.1 kb
359	22010-L21147	Exon 4b	696-697	CATCTACAAGCA-GTCACAGCACAT	0.2 kb
318	17421-L21315	Exon 5	795-796	TATCCGAGTGGA-AGGAAATTTGCG	0.7 kb
401	19650-L21141	Exon 6	891-892	CTCTGACTGTAC-CACCATCCACTA	0.5 kb
286 ‡	01999-L21411	Exon 7	1041-1042	CTGTCCTGGGAG-AGACCGGCGCAC	0.2 kb
391	17423-L21145	Exon 8	1155-1156	CTCTCCCCAGCC-AAAGAAGAAACC	0.2 kb
224 +	19638-L26297	Exon 9a	**NM_001126114.2**; 1196-1195 reverse	GCTGGTCTGGTC-CTTTAAAATATA	2.7 kb
346	17422-L21144	Exon 10	1248-1249	TGAGGCCTTGGA-ACTCAAGGATGC	1.0 kb
447	17424-L30914	Exon 11	1360-1361	CTCATGTTCAAG-ACAGAAGGGCCT	3.3 kb
175	19637-L26296	Downstream	2091 nt after exon 11 reverse	TGAAGCCATGAG-GAAATTGGGAGA	9.8 kb
		stop codon	*1382-1384 (exon 11)*		
382 ¬	19645-L26316	*ATP1B2* gene	17p13.1	AGAACCACCTTG-TCCTCAATTACA	5.0 kb
335 ¬	19884-L26749	*ATP1B2* gene	17p13.1	CCGCGCCACCAA-GATGGTCATCCA	65.6 kb
147 ¬	19643-L26685	*MPDU1* gene	17p13.1	GCTGCCCCAGGT-GTTTAAAATCCT	87.8 kb
459 ¬	09951-L30916	*POLR2A* gene	17p13.1	CGCCAAGTACAT-CATCCGAGACAA	1.8 kb
187 ¬	19647-L30910	*POLR2A* gene	17p13.1	GAAGACAATGAA-AGTTTTGCGCTG	

The *TP53* exon numbering used in this P056-D1 TP53 product description corresponds to the exon numbering from LRG_321, transcript t1 (NM_000546.5). Ligation sites of the *TP53* MLPA probes are indicated according to RefSeq sequence NM_000546.5 containing 11 exons, except when otherwise noted.

¬ 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.
‡ Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by IARC TP53 Database (http://p53.iarc.fr/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
+ Exon numbering for exon 9a is indicated according to LRG_321 transcript t3 (NM_001126114.2). Exon 9a is present in some alternative transcript variants only.

Table 2b. *CHEK2*

Length (nt)	SALSA MLPA probe	*CHEK2* exon[(a)]	Ligation site NM_007194.4	Partial sequence[(b)] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*59-61 (exon 2)*		
432 »	06631-L30915	Exon 8	923-924	TCATCAAGATTA-AAAACTTTTTTG	6.6 kb
154 »	21913-L06190	Exon 10	1079-1080	ACCTTCATGAAA-ACGGTATTATAC	1.1 kb
208 » §	18318-L26751	Exon 11	**1100delC** 1159-1157 reverse	TGCCCAAAATCA-TAATCTAAAATT	1.8 kb
181 » # «	21654-L30911	Exon 13	1509-1510	CTTAAGACACCC-GTGGCTTCAGGT	
		stop codon	*1688-1690 (exon 15)*		

The *CHEK2* exon numbering used in this P056-D1 TP53 product description is the exon numbering from LRG_302. Ligation sites of the CHEK2 MLPA probes are indicated according to RefSeq sequence NM_007194.4 containing 15 exons.

» Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P190.
§ Mutation-specific probe. This probe will only generate a signal when the *CHEK2* 1100delC mutation is present. Results obtained with this *CHEK2* 1100delC probe should be treated with caution! Please read the note on page 4.
« 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 probes 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.

Table 2c. Reference probes

Length (nt)	SALSA MLPA probe	Gene	Location (hg 18)	Partial sequence[(b)] (24 nt adjacent to ligation site)
372	14835-L26609	*UROD*	1p34	AAGCACCATGGC-TCAGGCCAAGCG
420	08839-L08899	*DYSF*	2p13	TGCCATGAAGCT-GGTGAAGCCCTT
480	21882-L15817	*EDAR*	2q13	AAAGCCCACCAA-GAGGTATGTGGA
135	16316-L21434	*RAB7A*	3q21	CACAATAGGAGC-TGACTTTCTGAC
129	19616-L26684	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG
193	11556-L26606	*MYOT*	5q31	CAGATCTCGGCT-ATACCTCCACCA
328	13397-L26608	*EYS*	6q12	ATGGTAAGATTA-ACTGAACCCTCT
247	08728-L08739	*PCSK5*	9q21	GACTATGAAGAA-TGTGTCCCTTGT
471	00979-L21316	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG
230	17130-L26574	*MYBPC3*	11p11	GTGCCTCAGTGA-CCAGGCTGGCTC
310	07028-L06639	*RPGRIP1*	14q11	GAGGTTCCCATT-GAAGCTGGCCAG
160	09787-L10202	*SPG11*	15q21	GGGACACATTCA-GGACTCAACAGA
274	17450-L30913	*GRIN2A*	16p13	TGCAGGATTATA-ATCTCACAATCT
490	19137-L25693	*PSMG1*	21q22	TGGAAGCTTTTA-AGCCTATACTTT

(a) The exon numbering and NM sequences used are from 09/2018, but can be changed by NCBI after the release of the product description.

(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.

Related SALSA MLPA probemixes

P190 CHEK2:	Contains probes for *CHEK2*, *ATM*, *TP53*, involved in breast cancer.
P002/P087 BRCA1	Contain probes for the *BRCA1* gene, involved in breast and ovarian cancer.
P045/P077/P090 BRCA2	Contain probes for the *BRCA2* gene, involved in breast and ovarian cancer.
P225 PTEN	Contains probes for the *PTEN* gene, involved in Cowden syndrome.

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P056 Product history	
Version	*Modification*
D1	Two flanking probes of TP53 and all flanking probes of CHEK2 have been removed. One CHEK2 probe has been added and one CHEK2 probe has been replaced. Moreover, nine probes have been changed in length but not in the sequence detected.
C1	Most of the reference and flanking probes are replaced and several are added. Furthermore, one probe for *TP53* exon 9a and one probe downstream *TP53* exon 11 are added. Moreover, one CHEK2 probe is replaced and one added.
B1	All reference probes and several target probes have been replaced and an additional probe for *TP53* exon 5 included. In addition, the 88 and 96 nt control fragments have been replaced.
A2	One reference probe has been replaced, five probes have a small change in length (but no change in sequence detected) and four extra control fragments at 88-96-100-105 nt have been included.
A1	Several new 17p probes have been added as compared to previous lots.
A	First release

Implemented changes in the product description

Version D1-02 – 05 October 2018 (04)
- Ligation sites of the probes targeting the *CHEK2* gene updated according to new version of the NM_reference sequence.
- Note was added under Table 1 and 2 for CHEK2 probes that have the same sequence as probes in the probemix P190.

Version D1-01 – 27 June 2018 (04)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- New references added.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- SD binning number changed from SD029 to SD067.
- Warning for SNPs 216nt and 256nt probes removed under Table 1 and 2.

Version 16 – 23 April 2015 (54)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new pictures included).
- Information added about the reference standard NG_017013.2, which is used for exon numbering.
- Various minor textual changes on the whole document.
- Electropherogram of the female reaction removed from page 7.
- SD information adjusted on page 2.

Version 15 – 02 February 2015 (54)
- Information about 1100delC mutation specific probe is added.

Version 14 (53)
- Reference added on page 2.
- Information about two probes (255 and 283 nt) added in Table 1 and Table 2.

Version 13 (53)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new pictures included)
- A note about exon numbering added below Table 1.
- Various minor textual changes on the whole document.

Version 12 (48)
- Five new references added on page 2.

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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

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