Product Description SALSA® MLPA® Probemix P190-D1 CHEK2

To be used with the MLPA General Protocol.

Version D1. Two CHEK2 probes have been added, the CHEK2 1100delC mutation probe has been replaced, all ATM probes have been replaced and twelve have been added, and two TP53 probes have been added. The PTEN, KLLN, XBP1 and BRCA1 probes have been removed. Moreover, almost all reference probes have been replaced and two have been added. Finally, several probes have a small change in length but no change in sequence detected. For complete product history see page 11.

Catalogue numbers:
- **P190-025R:** SALSA MLPA probemix P190 CHEK2, 25 reactions.
- **P190-050R:** SALSA MLPA probemix P190 CHEK2, 50 reactions.
- **P190-100R:** SALSA MLPA probemix P190 CHEK2, 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 P190 CHEK2 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *CHEK2*, *ATM* and *TP53* genes, and the detection of the *CHEK2* 1100delC variant, in order to determine increased susceptibility to breast cancer. An increased susceptibility to other cancer types, including but not limited to colon cancer, can also be determined with this probemix. This product can also be used for molecular genetic testing of at-risk family members.

Please note that this probemix covers all exons of *CHEK2* but not of *ATM* and *TP53*. For the latter two genes, the P041/P042 ATM and P056 TP53 probemixes provide a better coverage and may detect aberrations that are not detected by this P190 CHEK2 probemix.

This assay is for use with human DNA extracted from peripheral blood and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials. Deletions or duplications detected with the P190 CHEK2 probemix must be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in the aforementioned genes 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 these genes. 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: *CHEK2*, *ATM* and *TP53* all play important roles in DNA damage repair. A defect in one of these genes can lead to an increased risk of tumour formation. For breast cancer, autosomal dominant mutations in the genes *BRCA1* and *BRCA2* are the most frequent cause, followed by mutations in *CHEK2*, *ATM* and *PALB2*, though with a much lower frequency (Buys et al. 2017). Mutations in *CHEK2* may also increase the risk of developing colorectal cancer (Xiang et al. 2011) and other cancers, including prostate cancer (Cybulski et al. 2006). Mutations in *CHEK2*, and the 1100delC mutation in particular, have also been suggested as an underlying cause of Li-Fraumeni syndrome (LFS) type 2. Moreover, a deletion in

CHEK2 was found in a patient fulfilling Li-Fraumeni-Like (LFL) criteria (Ruijs et al. 2009). Researchers are uncertain whether *CHEK2* mutations actually cause LFS or are merely associated with an increased risk of several types of cancer, including cancers seen in LFS. For more information: https://omim.org/entry/609265.

Exons 11-15 of *CHEK2* share a high sequence homology with several *CHEK2* pseudogenes, which can result in a pseudogene-mediated gene conversion (Pan et al. 2017).

Autosomal dominant mutations in *ATM* are linked to an increased risk of cancer, with breast cancer in particular (see Table 1). Autosomal recessive mutations of *ATM* cause Ataxia-Telangiectasia, which is characterized by progressive cerebellar ataxia, telangiectases, and a predisposition to malignancy, particularly leukaemia and lymphoma. For more information: https://www.ncbi.nlm.nih.gov/books/NBK26468/.

Autosomal dominant *TP53* mutations result in LFS. The most common types of tumours in LFS are soft tissue sarcomas and osteosarcomas, pre-menopausal breast cancer, brain tumours, leukaemia, and adrenocortical carcinoma. Families presenting incomplete features of LFS are referred to as having LFL, and around 20-40% of these patients have a germline mutation in *TP53* (Ruijs et al. 2010). More information on LFS: https://www.ncbi.nlm.nih.gov/books/NBK1311/.

Table 1. Overview of different types of cancer caused by mutations in *CHEK2, ATM* and *TP53*, and the associated relative risks of developing these cancers.

Gene	Cancer	Relative risk	Occurrence mutations*	References[#]
CHEK2	Breast	Lifetime risk of 25-39% in heterozygotes, though this is dependent on the variant and family history	0-3.5% depending on ethnicity	PMID: 18172190, 21876083, 18381420, 28085182, 15122511, 27595995
	Other cancers, such as colorectal and prostate	Only preliminary evidence	Not known	PMID: 21807500, 25431674, 17085682, 24506336, 22901170
ATM	Breast	Lifetime risk of 17-52%, but can be dependent on the variant	~1%	PMID: 26523341, 28085182 16832357, 19781682, 22585167, 27595995
	Other cancers, such as pancreatic, ovarian and prostate	Only preliminary evidence	Not known	PMID: 22585167, 26483394, 27433846, 18565893, 29348823, 29486991
TP53	(Pre-menopausal) Breast	Lifetime risk of ~79%	<1%	PMID: 20522432, 28085182, 25467110, 26523341
	Other cancers, such as sarcomas and brain tumours	Lifetime risk of cancer in general for men is 73%; for women this is nearly 100%	~80% of families with features of LFS	PMID: 10864200, 20522432

* Including point mutations, indels, deletions and duplications. Percentages depend on the population tested. For example, these percentages may be higher in a *BRCA1/2* mutation negative population.
[#] PMID: PubMed unique identifier.

Gene structure:

- The *CHEK2* gene (NG_008150.2) spans 54 kilobases (kb) on chromosome 22q12.1 and has 15 exons. The *CHEK2* LRG_302 is pending approval and is available at www.lrg-sequence.org.
- The *ATM* gene spans ~146 kb on chromosome 11q22.3 and has 63 exons. The *ATM* LRG_135 is available and identical to NG_009830.1.

- The *TP53* gene spans ~19 kb on chromosome 17p13.1 and has 11 exons. The *TP53* LRG_321 is available and identical to NG_017013.2.

Transcript variants:
- For *CHEK2*, multiple transcript variants have been described of which transcript variant 1 (NM_007194.4; 1844 nt; coding sequence 59-1690; https://www.ncbi.nlm.nih.gov/gene/11200) represents the predominant transcript and encodes isoform a. 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 15.
- For *ATM,* multiple transcript variants have been described of which transcript variant 2 (NM_000051.3; 13147 nt; coding sequence 386-9556; https://www.ncbi.nlm.nih.gov/gene/472) is a reference standard in the RefSeqGene project. The ATG start site is located in exon 2 and the stop codon is located in exon 63.
- For *TP53*, multiple transcript variants have been described 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 2a and the stop codon in exon 11.

Probemix content: This SALSA MLPA probemix P190 CHEK2 contains 53 probes with amplification products between 124 and 500 nt (Table 2), including ten reference probes. At least one probe is present for each exon of *CHEK2* and one probe is included just upstream of *CHEK2.* Moreover, there is one probe specific for the *CHEK2* 1100delC mutation, which will only generate a signal when this mutation is present. Nineteen probes for *ATM* and four probes for *TP53* are also present. These probes target sequences in various parts of the genes, including in the first and last exon. 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 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).

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 peripheral blood, 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 hereditary 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 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, HG00343, NA08618 and HG03694 from the Coriell Institute have been tested at MRC-Holland and can be used as positive control samples. NA07106 has a partial trisomy of chromosome 22, including a heterozygous *CHEK2* duplication, and HG00343 has a *CHEK2* exon 9-10 deletion. NA08618 has a partial duplication of the 11q arm, which includes the whole *ATM* gene, and HG03694 has an *ATM* exon 62-63 duplication. In addition, Coriell sample HG00187 (1000 genomes) can be used as a positive control sample for the *CHEK2* 1100delC mutation. The quality of cell lines can change; therefore, samples should be validated before use.

SALSA Binning DNA SD078: The SD078 Binning DNA provided with this probemix can be used as Binning DNA sample for binning of one mutation-specific probe (313 nt probe 22034-SP0468-L31261 *CHEK2* 1100delC mutation). SD078 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD078 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/SNP 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 SD078 Binning DNA product description.

Performance characteristics:

- Deletions or duplications in *CHEK2* are rare (Ruijs et al. 2009, Tedaldi et al. 2014, Havranek et al. 2015, Apostolou et al. 2018), although the exon 9/10 deletion is found more frequently (~1.0%) in Slavic populations (Walsh et al. 2006, Cybulski et al. 2007). The overall prevalence of the *CHEK2* 1100delC mutation in breast cancer is ~0.9%, although the mutation has a higher prevalence in patients with a family history of breast cancer or patients from Northern European origin (Zhang et al. 2008).
- The frequency of *ATM* deletions or duplications in breast cancer is less than 0.1% (Tung et al. 2015, Susswein et al. 2016). Please note that only 19 out of 63 exons are covered in this probemix, which means that not all deletions or duplications can be detected with this probemix.
- The frequency of deletions or duplications in *TP53* in Li-Fraumeni syndrome or Li-Fraumeni-like is ~1% in *TP53* point mutation negative patients (https://www.ncbi.nlm.nih.gov/books/NBK1311/, Mouchawar et al. 2010, Schulz et al. 2012). In breast cancer patients without an indication for Li-Fraumeni syndrome, the frequency will be lower (Mouchawar et al. 2010, Susswein et al. 2016). Moreover, only 4 out of 11 exons are covered in this probemix, which means that not all deletions or duplications can be detected.

These percentages are dependent on the population tested. For example, these percentages may be higher in a population of *BRCA1/2* mutation negative patients.

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 2008-2017 literature review).
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 *CHEK2*, *ATM* and *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 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

- 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 reference probes or only the flanking probe are unlikely to have any relation to the condition tested for.

Notes on the P190 probemix:

- **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. Results obtained with this CHEK2 mutation probe should therefore be treated with caution, in particular when a signal is observed in all samples in an experiment.
- Deletions of the last exons of *ATM* (exon 62-63) are encountered relatively frequently (own validation observations, Micol et al. 2011, Nakamura et al. 2012, Podralska et al. 2014, Tung et al. 2015, Susswein et al. 2016). *Duplication* of these exons is suggested not to be associated with an increased risk of hereditary breast cancer (LaBreche et al. 2017). Therefore, duplication of the last exons of *ATM* should be interpreted with caution.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *CHEK2, ATM* or *TP53* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P190 CHEK2.

- Not all exons of *ATM* and *TP53* are covered. 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: The SALSA MLPA probemixes P041/P042 ATM can be used for confirmation of the *ATM* results and to further analyse potential *ATM* deletions and duplications. When P041/P042 ATM are used together, every exon of *ATM* is covered by a probe. For *TP53*, SALSA MLPA probemix P056 TP53 can be used for confirmation of results and to further analyse potential *TP53* deletions and duplications. This probemix has a probe for each exon of *TP53*.
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 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.

Mutation databases:
For *CHEK2*: http://grenada.lumc.nl/LSDB_list/lsdbs/CHEK2 or https://www.ncbi.nlm.nih.gov/clinvar.
For *ATM*: http://chromium.lovd.nl/LOVD2/home.php.
For *TP53*: http://p53.iarc.fr/ or http://grenada.lumc.nl/LSDB_list/lsdbs/TP53.
We strongly encourage users to deposit positive results in the corresponding database. 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 *CHEK2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 2. SALSA MLPA Probemix P190-D1 CHEK2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a]			
		Reference	*CHEK2*	*ATM*	*TP53*
64-105	Control fragments – see table in probemix content section for more information				
124 *	Reference probe 18709-L21056	5q31			
131 *	**ATM probe** 21650-L30272			**Exon 58**	
136 *	**ATM probe** 21651-L30273			**Exon 43**	
143 ¥	**CHEK2 probe** 21419-L29916		**Exon 12**		
148 *	Reference probe 14199-L23450	2q13			
154	**CHEK2 probe** 06630-L07119		**Exon 7**		
160 *	**ATM probe** 21652-L30274			**Exon 38**	
166 *	**TP53 probe** 21653-L30275				**Exon 8**
172 ¥	**CHEK2 probe** 21418-L29915		**Exon 11**		
178 *	**CHEK2 probe** 21654-L30276		**Exon 13**		
184 *	**ATM probe** 21655-L30277			**Exon 55**	
190 *	**ATM probe** 21656-L30840			**Exon 63**	
196 *	**ATM probe** 21657-L30279			**Exon 24**	
202 *	**ATM probe** 21658-L30280			**Exon 61**	
208 ¥	**CHEK2 probe** 06629-L30453		**Exon 6**		
214 *	Reference probe 10730-L30523	6p12			
220 ¥	**CHEK2 probe** 06623-L31306		**Promoter**		
226 ¥	**TP53 probe** 01997-L31312				**Exon 3**
232 *	**ATM probe** 21659-L30281			**Exon 20**	
238 *	**ATM probe** 21660-L30282			**Exon 9**	
244 ¥	**CHEK2 probe** 06636-L30524		**Exon 14**		
250 *	Reference probe 17871-L22467	2p21			
256 ¥ Ж	**CHEK2 probe** 07281-SP0890-L30457		**Exon 15**		
263 *	**ATM probe** 21661-L30283			**Exon 49**	
268 ¥ ¬	**HSCB probe** 06800-L30458		**Upstream**		
274	**CHEK2 probe** 06627-L06185		**Exon 4**		
281 *	**TP53 probe** 21581-L25982				**Exon 1**
286 *	**ATM probe** 21662-L30284			**Exon 17**	
292 *	**ATM probe** 21663-L30525			**Exon 8**	
298 *	Reference probe 15388-L17790	3p22			
306 ¥	**CHEK2 probe** 06626-L30841		**Exon 3**		
313 * Ж § » П	**CHEK2 probe** 22034-SP0468-L31261		**1100delC**		
319 *	**ATM probe** 21664-L30286			**Exon 33**	
328 *	**ATM probe** 21665-L30287			**Exon 1**	
337 »	**CHEK2 probe** 06624-L24131		**Exon 1**		
346 ¥	**TP53 probe** 00345-L31314				**Exon 11**
355	**CHEK2 probe** 19654-L26320		**Exon 13**		
364 *	Reference probe 18676-L24030	11p14			
373	**CHEK2 probe** 06628-L06186		**Exon 5**		
382 *	**CHEK2 probe** 21666-L30288		**Exon 14**		
391	**CHEK2 probe** 06625-L06183		**Exon 2**		
400 »	**CHEK2 probe** 02579-L02041		**Exon 9**		
409	Reference probe 08725-L08736	9q21			
418 П	**CHEK2 probe** 06632-L06190		**Exon 10**		
427 П	**CHEK2 probe** 06631-L06189		**Exon 8**		
436 *	**ATM probe** 21668-L30290			**Exon 5**	
445 *	**ATM probe** 21669-L30291			**Exon 45**	
454 *	Reference probe 18691-L02476	5p15			
463 *	**ATM probe** 21670-L30292			**Exon 62**	
474 *	**ATM probe** 21671-L30293			**Exon 29**	
483 *	**ATM probe** 21672-L30294			**Exon 15**	
490 *	Reference probe 20096-L27538	4p15			
500 *	Reference probe 17001-L22947	20q11			

(a) The *CHEK2* exon numbering is identical to the LRG_302 sequence. The *ATM* exon numbering is from the RefSeq transcript NM_000051.3, which is identical to the LRG_135 sequence. The *TP53* exon numbering is from RefSeq transcript NM_000546.5, which is identical to the LRG_321 sequence. The exon numbering and NM sequences used are from 08/2018, but can be changed by NCBI after the release of the product description.

* New in version D1 (from lot D1-0718 onwards).
¥ Changed in version D1 (from lot D1-0718 onwards). Small change in length, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
¬ 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.
§ Mutation-specific probe. This probe will only generate a signal when the *CHEK2* 1100delC mutation is present.
» Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P045.
Π Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P056.

Table 3. P190 probes arranged according to chromosomal location

Table 3a. *CHEK2* at 22q12.1

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
268 ¬	06800-L30458	*HSCB* gene		TGGCTGAAGAAA-TCTGGGTGGACA	1.9 kb
		start codon	*59-61 (exon 2)*		
220	06623-L31306	Promoter	208 nt before exon 1	CTAACCAGACTA-ATGTTGCTGATT	0.2 kb
337 »	06624-L24131	Exon 1	3-4	TTTAGCGCCACT-CTGCTGGCTGAG	7.3 kb
391	06625-L06183	Exon 2	270-271	CACTCAGGAACT-CTATTCTATTCC	9.2 kb
306	06626-L30841	Exon 3	449-450	AACCACTGCTGA-AAAGAACAGATA	0.2 kb
274	06627-L06185	Exon 4	571-572	ACCTTTGTAAAT-ACAGAGCTTGTA	5.6 kb
373	06628-L06186	Exon 5	704-705	ATCCTAAGGCAT-TAAGAGATGAAT	7.5 kb
208	06629-L30453	Exon 6	780-781	TTTCGAGAGGAA-AACATGTAAGAA	2.0 kb
154	06630-L07119	Exon 7	871-872	CTCAATGTTGAA-ACAGAAATAGAA	6.5 kb
427 Π	06631-L06189	Exon 8	923-924	TCATCAAGATTA-AAAACTTTTTTG	3.6 kb
400 »	02579-L02041	Exon 9	994-995	CTGTTTGACAAA-GTGGTGGGGAAT	2.9 kb
418 Π	06632-L06190	Exon 10	1079-1080	ACCTTCATGAAA-ACGGTATTATAC	1.1 kb
313 § Ж » Π	22034-SP0468-L31261	Exon 11	1159-1157 and 38 nt before exon 11 reverse	TGCCCAAAATCA-42nt spanning oligo-CATAAAATAAAA	0.2 kb
172 #	21418-L29915	Exon 11	21 nt after exon 11 reverse	ACCAGTCTGTGC-AGCAATGAAAAT	0.6 kb
143 #	21419-L29916	Exon 12	46 nt after exon 12 reverse	ACCACAGCACAT-ACACATTTTAGC	0.9 kb
355 #	19654-L26320	Exon 13	54 nt before exon 13	TCTGGCATACTC-TTACTGATAATA	0.1 kb
178 Π #	21654-L30276	Exon 13	1509-1510	CTTAAGACACCC-GTGGCTTCAGGT	4.7 kb
382 #	21666-L30288	Exon 14	96 nt before exon 14	TGGACGGACATT-TTTCCTCCCTCT	0.4 kb
244 #	06636-L30524	Exon 14	207 nt after exon 14 reverse	CTGTGCTTATCG-GTCTATTATGTG	1.0 kb
256 # Ж	07281-SP0890-L30457	Exon 15	1624-1625 and 1666-1667	CGAAAGCGGCCC-42nt spanning oligo-GCTGTGTGTGCT	
		stop codon	*1688-1690 (exon 15)*		

The exon numbering used in this P190 CHEK2 product description for *CHEK2* is the exon numbering identical to LRG_302. Ligation sites of the CHEK2 MLPA probes are indicated according to RefSeq sequence NM_007194.4 containing 15 exons.

¬ 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 P045.
Π Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P056.
§ Mutation-specific probe. This probe will only generate a signal when the *CHEK2* 1100delC mutation is present.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.

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.

Table 3b. *ATM* at 11q22.3

Length (nt)	SALSA MLPA probe	*ATM* exon[a]	Ligation site NM_000051.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*386-388 (exon 2)*		
328	21665-L30287	Exon 1	172-173	GGCTGCTTGGCG-TTGCTTCTTCCT	12.8 kb
436	21668-L30290	Exon 5	814-815	GATTGTAGCAAC-ATACTACTCAAA	11.2 kb
292	21663-L30525	Exon 8	1317-1318	ATGGAGAAGTAT-TTTATACAACTT	2.1 kb
238	21660-L30282	Exon 9	1575-1576	GGAAGTAATAAA-AGATCACCTTCA	8.5 kb
483	21672-L30294	Exon 15	2657-2658	AATGTGCAGGAG-AAAGTATCACTC	9.8 kb
286	21662-L30284	Exon 17	2967-2968	ATTTAACGATTA-CCCTGATAGTAG	4.0 kb
232	21659-L30281	Exon 20	3361-3362	ACTATTTTAAAC-CATGTCCTTCAT	9.8 kb
196	21657-L30279	Exon 24	3934-3935	GTGAAAGAGAAT-GGATTAGAACCT	8.5 kb
474	21671-L30293	Exon 29	4628-4629	TCTAGGATTCCT-ATCAGAAAATTC	7.7 kb
319	21664-L30286	Exon 33	5325-5326	TATGGTGAAACT-AGTTGTCAATTT	10.6 kb
160	21652-L30274	Exon 38	6120-6121	AATGCTTGCTGT-TGTGGACTACAT	9.5 kb
136	21651-L30273	Exon 43	6704-6705	CATGGAGGAATA-TGCAGTGGGACC	3.8 kb
445	21669-L30291	Exon 45	6869-6870	TGTGTAAGCGCA-GCCTTGAGTCTG	7.7 kb
263	21661-L30283	Exon 49	7494-7495	AGTTGCTGGAAA-TTATGATGGAGA	6.0 kb
184	21655-L30277	Exon 55	8500-8501	ATAGATTGTGTA-GGTTCCGATGGC	10.8 kb
131	21650-L30272	Exon 58	8933-8934	TTGAGAAGCGAT-TGGCTTATACGC	9.0 kb
202	21658-L30280	Exon 61	9225-9226	AACTCTGTTAAC-CATTGTAGAGGT	10.3 kb
463	21670-L30292	Exon 62	9270-9271	TGACTGGACCAT-GAATCCTTTGAA	0.3 kb
190	21656-L30840	Exon 63	9514-9515	ATAGACCCCAAA-AATCTCAGCCGA	
		stop codon	*9554-9556 (exon 63)*		

The exon numbering used in this P190 CHEK2 product description for *ATM* is the exon numbering from the RefSeq transcript NM_000051.3, which is identical to LRG_135. Ligation sites of the ATM MLPA probes are indicated according to RefSeq sequence NM_000051.3 containing 63 exons.

Table 3c. *TP53* at 17p13.1

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
		start codon	*203-205 (exon 2a)*		
281	21581-L25982	Exon 1	52-51 reverse	GAGAAGCTCAAA-ACTTTTAGCGCC	11.2 kb
226	01997-L31312	Exon 3	301-302	CATCTACAGTCC-CCCTTGCCGTCC	2.7 kb
166	21653-L30275	Exon 8	1184-1185	ATGGAGAATATT-TCACCCTTCAGG	3.9 kb
346	00345-L31314	Exon 11	1329-1330	AAAGGGTCAGTC-TACCTCCCGCCA	
		stop codon	*1382-1384 (exon 11)*		

The exon numbering used in this P190 CHEK2 product description for *TP53* is the exon numbering from the RefSeq transcript NM_000546.5, which is identical to LRG_321. Ligation sites of the TP53 MLPA probes are indicated according to RefSeq sequence NM_000546.5 containing 11 exons.

(a) The exon numbering and NM sequences used are from 08/2018, 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

P002 BRCA1	Contains probes for *BRCA1*, involved in breast and ovarian cancer.
P087 BRCA1 Confirmation	Contains probes for *BRCA1*, as confirmation of the P002 probemix.
P045 BRCA2/CHEK2	Contains probes for *BRCA2* and *CHEK2*, involved in breast and ovarian cancer.
P090 BRCA2	Contains probes for *BRCA2*, involved in breast and ovarian cancer.

P077 BRCA2 Confirmation	Contains probes for *BRCA2*, as confirmation of the P090/P045 probemixes.
P041/P042 ATM	Contain probes for the *ATM* gene, involved in breast cancer and Ataxia Telangiectasia.
P056 TP53	Contains probes for the *TP53* gene, involved in Li-Fraumeni syndrome.
P260 PALB2-RAD50-RAD51C-RAD51D	Contains probes for *PALB2, RAD50, RAD51C* and *RAD51D*, involved in breast and ovarian cancer.

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P190 Product history	
Version	*Modification*
D1	Two CHEK2 probes have been added, the CHEK2 1100delC mutation probe has been replaced, all ATM probes have been replaced and twelve have been added, and two TP53 probes have been added. The PTEN, KLLN, XBP1 and BRCA1 probes have been removed. Moreover, almost all reference probes have been replaced and two have been added. Finally, several probes have a small change in length but no change in sequence detected.
C1	One ATM probe has been removed, several probes have been adjusted and four reference probes have been replaced.
B2	New control fragments have been added (QDX2) and three probes have been elongated with no change in hybridising sequence.
B1	All reference probes have been replaced. The BRCA2 probe at 136 nt has been removed.
A1	First release.

Implemented changes in the product description

Version D1-01 – 05 October 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 2 and Table 3).
- Clinical background was rewritten and Table 1 was added.
- SD binning DNA has changed.
- Warning for SNP for probe 07281-SP0890-L30457 was removed.
- Ligation sites of the probes targeting the *CHEK2* gene updated according to new version of the NM_reference sequence.
- Warning added to Table 3 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 21 – 15 September 2017 (55)
- Warning added in Table 1 and Table 2, 246 nt probe 05162-L04543.
- Various minor textual changes throughout the document.

Version 20 – 16 August 2016 (55)
- Product description adapted to new lot number (lot number changed, new picture included).

Version 19 – 16 August 2016 (55)
- 265 nt probe for HSCB gene is no longer present in P045.
- Manufacturer's address adjusted.

Version 18 – 09 March 2015 (54)
- Product description adapted to new lot number (lot number changed, new picture included
- Various minor textual changes.

Version 17 – 02 February 2015 (54)
- Information about 1100delC mutation specific probe is added.

Version 16 (53)
- Product description adapted to a new product version (version number changed, lot number added,

changes in Table 1 and Table 2, new picture included).

Version 15 (51)

- CHEK2 exon numbering changed according to NM_007194.3, reference standard in the RefSeqGene project.
- Small textual changes on page 1 and 2.

Version 14 (51)

- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

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