Product Description SALSA® MLPA® Probemix P045-D1 BRCA2/CHEK2

To be used with the MLPA General Protocol.

Version D1. As compared to version C1, the BRCA2 exon 3/ c.156_157insALU probe has been changed from a 3-part to a 2-part probe in order to reduce its sensitivity to sample DNA depurination. One probe has a small change in length, but not in sequence detected. One reference probe has been replaced. For complete product history see page 12.

Catalogue numbers:

- **P045-025R:** SALSA MLPA Probemix P045 BRCA2/CHEK2, 25 reactions.
- **P045-050R:** SALSA MLPA Probemix P045 BRCA2/CHEK2, 50 reactions.
- **P045-100R:** SALSA MLPA Probemix P045 BRCA2/CHEK2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mlpa.com).

There are three probemixes available for *BRCA2* testing at MRC-Holland. Content and use is described below:

Probemix	Gene 1	Gene 2	Coverage	Use for	Remarks
P045 BRCA2/CHEK2	*BRCA2*	*CHEK2*	*BRCA2:* Each exon *CHEK2:* Exon 1, 9, 1100delC mutation (exon 11)	Initial testing	Identical BRCA2 probes as P090
P090 BRCA2	*BRCA2*	-	Each exon	Initial testing	Identical BRCA2 probes as P045
P077 BRCA2 Confirmation	*BRCA2*	-	Each exon	Confirmation	BRCA2 probes target different ligation sites than probes in P090/P045

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 P045 BRCA2/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 *BRCA2* gene and the presence of the c.156_157insAlu mutation, in order to confirm a potential cause and clinical diagnosis for hereditary breast and ovarian cancer (HBOC) or in rare cases Fanconi Anemia type D1. In addition, the 1100delC mutation and deletions or duplications of exon 1 and 9 in *CHEK2* can be detected, in order to determine predisposition to breast cancer, but also other cancer types, including but not limited to colon and prostate cancer. This product can also be used for molecular genetic testing of family members who are at risk for the mentioned aberrations.

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 P045 BRCA2/CHEK2 probemix must be verified by using the SALSA MLPA probemix P077 BRCA2 Confirmation or a different technique. P077 BRCA2 Confirmation cannot be used to verify *CHEK2* mutations. However, the P190 CHEK2 probemix is available for deletion or duplication analysis of other *CHEK2* exons. Most defects in the *BRCA2* gene are point mutations, the majority of which will not be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of the

BRCA2 gene. This assay 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 (IVD) use 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: Breast and ovarian carcinomas are among the most common malignancies in developed countries. The majority of cases are considered sporadic, but in a substantial portion, a clear history of cases within a family is present. The BRCA1 and BRCA2 proteins are associated with the activation of double-strand break repair and homologous recombination and are important in maintaining genomic stability. Germline mutations in the *BRCA1* and *BRCA2* genes are linked to a high risk of young-onset hereditary breast and ovarian cancer. Features characteristic for hereditary, versus sporadic, breast cancer are: younger age at diagnosis, frequent bilateral disease, and more frequent occurrence of disease among male relatives. Mutations in the *BRCA1* and *BRCA2* genes account for about 20 to 25% of hereditary breast cancers (Easton 1999) and about 5 to 10% of all breast cancers (Campeau et al. 2008). In addition, mutations in the *BRCA1* and *BRCA2* genes cause around 15% of ovarian cancers overall (Pal et al. 2005).

Deletions or duplications are more frequent for *BRCA1* than for *BRCA2* in most populations. The prevalence of deletions or duplications is dependent on the studied population and ranges from 0% to 11% of all *BRCA2* mutations (Agata et al. 2005, Woodward et al. 2005, Casilli et al. 2006, Stadler et al. 2010).

More information is available at http://www.ncbi.nlm.nih.gov/books/NBK1247/.

Biallelic pathogenic variants of *BRCA2* can result in Fanconi Anemia (FA) type D1. FA is characterized by physical abnormalities (such as short stature or abnormal skin pigmentation), bone marrow failure and increased risk for malignancies. The incidence of FA in general is 1:160,000, of which type D1 comprises around 3% of the cases. FA type D1 is associated with early-onset acute leukaemia and solid tumours. More information on FA is available at https://www.ncbi.nlm.nih.gov/books/NBK1401/.

BRCA1 and *BRCA2* mutations are most frequently found, but other genes are also associated with an increased risk for developing breast and ovarian cancer, including *CHEK2*. The protein CHK2 is a cell cycle checkpoint regulator and a putative tumour suppressor. In non-BRCA1/2 breast cancer families, patients heterozygous for the *CHEK2* 1100delC mutation have a two times increased risk of developing breast cancer and have a higher contralateral breast cancer rate (Huijts et al. 2014, Kriege et al. 2014). A deletion of exon 9 and 10 in *CHEK2* has been found mainly in Slavic populations and is associated with a two times higher risk for breast cancer (Walsh et al. 2006).

Gene structure: The *BRCA2* gene spans 84 kilobases (kb) on chromosome 13q13.1 and contains 27 exons. The *BRCA2* LRG_293 is available at www.lrg-sequence.org and is identical to GenBank NG_012772.3.
The *CHEK2* gene is located on chromosome 22q12.1 (reverse strand), spans 54 kb and contains 22 exons. The preliminary *CHEK2* LRG_302 is still pending final approval and is identical to GenBank NG_008150.1.

Transcript variants: For *BRCA2,* one transcript variant has been described encoding the full length protein (NM_000059.3; 11386 nt; coding sequence 228-10484; http://www.ncbi.nlm.nih.gov/gene/675). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2 and the stop codon is located in exon 27.
For *CHEK2* multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform *a* (NM_007194.4; 1844 nt; coding sequence 59-1690).

Exon numbering: The *BRCA2* exon numbering used in this P045-D1 BRCA2/CHEK2 product description is the exon numbering from the RefSeq transcript NM_000059.3, which is identical to the LRG_293 sequence. For *CHEK2*, the exon numbering is identical to NG_008150.2. The exon numbering and NM_ sequence used have been retrieved on 01/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P045-D1 BRCA2/CHEK2 contains 51 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 40 probes for the *BRCA2* region and three probes for the *CHEK2* region. In addition, eight reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

At least one MLPA probe is present for each exon in the *BRCA2* transcript; two probes are present for exons 1 and 3, three probes are present for exons 10 and 27, and six probes are present for exon 11. One of the probes for exon 3 detects the wild type sequence of the c.156_157insAlu mutation and a reduced signal can point towards the presence of this mutation **or** a deletion of exon 3. In addition, there is a probe for a sequence upstream and a probe for a sequence downstream of *BRCA2*.

For the *CHEK2* gene, one probe is present for exons 1 and 9. Moreover, one probe specific for the *CHEK2* 1100delC mutation is included, which will only generate a signal when the mutation is present.

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA 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: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of hereditary predisposition to 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 Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID numbers NA03330 and NA02718 from the Coriell Institute have been tested with this P045-D1 probemix at MRC-Holland and can be used as positive control samples. NA03330 contains DNA with a trisomy of chromosome 13, which includes a whole gene duplication of *BRCA2*, whereas NA02718 contains DNA with a partial deletion of chromosome 13q resulting in a whole gene deletion of *BRCA2*.

In addition, Coriell sample HG00187 (1000 Genomes Project) 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 SD067: The SD067 Binning DNA provided with this probemix can be used for binning of the *CHEK2* 1100delC mutation-specific probe (490 nt probe 01772-L01336). 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/SNP positive patient samples or cell lines should be used. It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD067 Binning DNA product description, available online: www.mlpa.com.

SALSA Artificial Duplication DNA SD024: In case no positive DNA sample is available in your laboratory, an artificial duplication DNA sample for this probemix (catalogue number SD024) can be ordered from MRC-Holland. This SD024 Artificial Duplication DNA will show a duplication of two or more probes when using the following probemixes: P045, P090 and P077 for *BRCA2* and P002 and P087 for *BRCA1.* The SD024 Artificial Duplication DNA is a mixture of human female genomic DNA and a titrated amount of plasmid containing selected probe target sequences. For further details, please consult the SD024 Artificial Duplication DNA product description, available online: www.mlpa.com. **This product is for research use only (RUO).**

Performance characteristics: The frequency of *BRCA2* deletions or duplications in hereditary breast and ovarian cancer families is ~1%, dependent on the population (Walsh et al. 2006, http://www.ncbi.nlm.nih.gov/books/NBK1247/). No deletions or duplications for *BRCA2* have been described for FA type D1. Deletions or duplications in *CHEK2* are rare, whereas the overall prevalence of the *CHEK2* 1100delC mutation in breast cancer is around 0.9%, depending on ethnicity (Zhang et al. 2008). The analytical sensitivity and specificity for the detection of deletions or duplications in the *BRCA2* gene and *CHEK2* exons 1 and 9 in samples without mutations in *BRCA2* or *CHEK2*, is very high and can be considered >99% (based on a 2011-2018 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 should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The expected results for the *BRCA2* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), and occasionally 4 (homozygous duplication or heterozygous triplication, e.g. Judkins et al. 2012). A homozygous deletion (copy number 0) of the *BRCA2* gene is unlikely, but may result in FA type D1 or be embryonically lethal (Loizidou et al. 2016).

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

P045 specific note:

- **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.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *BRCA2* or *CHEK2* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P045 BRCA2/CHEK2.
- 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.
- Several (putative) founder mutations for *BRCA2* have been described, which can cause false positive results (see limitation above). This includes the *BRCA2* 999del5 (rs80359671) Finnish/Icelandic founder mutation (Hartikainen et al. 2007) in the BRCA2 exon 9 probe.

Confirmation of results: Deletions or duplications obtained with the P045 BRCA2/CHEK2 probemix must be verified by using the SALSA MLPA probemix P077 BRCA2 Confirmation or a different 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. All probes included in SALSA MLPA probemix P077 BRCA2 Confirmation are different from those in probemix P045 BRCA2/CHEK2 or probemix P090 BRCA2. The c.156_157insAlu mutation must be verified with another method, such as nested PCR (Machado et al. 2007). The P190 CHEK2 probemix can be used to further analyse potential deletions and duplications in the *CHEK2* gene.

***BRCA1/2* mutation databases:** http://research.nhgri.nih.gov/bic/; http://BRCA1.lovd.nl; http://BRCA2.lovd.nl. We strongly encourage users to deposit positive results in the Breast Cancer Mutation Databases. 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 *BRCA2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P045-D1 BRCA2/CHEK2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	BRCA2	CHEK2
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L00463	5q31		
136	**BRCA2 probe** 02283-L26707		**Exon 1**	
142	**BRCA2 probe** 18385-L23778		**Exon 11**	
149	**BRCA2 probe** 20546-L28140		**Exon 19**	
154	**BRCA2 probe** 02285-L23744		**Exon 1**	
160	**BRCA2 probe** 09297-L28129		**Exon 14**	
166	**BRCA2 probe** 20603-L28261		**Exon 11**	
172	**BRCA2 probe** 02486-L23747		**Exon 2**	
178	Reference probe 04532-L03921	2q24		
184	**BRCA2 probe** 20625-L28317		**Exon 22**	
190	**BRCA2 probe** 18387-L24251		**Exon 11**	
196	**BRCA2 probe** 09812-L23750		**Exon 23**	
202	**BRCA2 probe** 01600-L23751		**Exon 4**	
208	**BRCA2 probe** 08265-L23752		**Exon 7**	
214	Reference probe 11996-L12824	6q25		
220	**BRCA2 probe** 18388-L23375		**Exon 10**	
226	**BRCA2 probe** 20626-L28778		**Exon 25**	
232	**BRCA2 probe** 01603-L13850		**Exon 9**	
238 ¤ ∞	**BRCA2 probe** 22219-L31553		**Exon 3/ c.156_157insAlu**	
244 ¥ ¬	**BRCA2 probe** 20548-L31554		**Upstream**	
250	**BRCA2 probe** 01604-L23754		**Exon 10**	
257	Reference probe 02469-L28780	15q21		
265	**BRCA2 probe** 20549-L28781		**Exon 11**	
271 »	**CHEK2 probe** 20724-L29194			**Exon 1**
275	**BRCA2 probe** 18389-L24255		**Exon 27**	
283	**BRCA2 probe** 01606-L23757		**Exon 11**	
291	**BRCA2 probe** 20676-L28319		**Exon 18**	
295	**BRCA2 probe** 20541-L28782		**Exon 27**	
304	Reference probe 11441-L28327	1q41		
313	**BRCA2 probe** 02280-L28326		**Exon 13**	
321	**BRCA2 probe** 09809-L28325		**Exon 5**	
328	**BRCA2 probe** 19699-L28324		**Exon 27**	
337	**BRCA2 probe** 20628-L28320		**Exon 12**	
346	**BRCA2 probe** 01611-L23763		**Exon 16**	
355	**BRCA2 probe** 04585-L23764		**Exon 6**	
364	**BRCA2 probe** 02281-L23765		**Exon 17**	
373	**BRCA2 probe** 20629-L28321		**Exon 21**	
382	Reference probe 13329-L14755	18q21		
391	**BRCA2 probe** 20543-L28130		**Exon 10**	
400	**BRCA2 probe** 08266-L23768		**Exon 20**	
409 Ж »	**CHEK2 probe** 02579-L23769			**Exon 9**
418	**BRCA2 probe** 20630-L28322		**Exon 15**	
426	**BRCA2 probe** 20631-L25993		**Exon 3**	
436	Reference probe 07975-L07756	17q21		
445	**BRCA2 probe** 08267-L23772		**Exon 24**	
454	**BRCA2 probe** 20632-L28323		**Exon 8**	
462 ¬	**N4BP2L1 probe** 18948-L01619		**Downstream**	
472	**BRCA2 probe** 11984-L23775		**Exon 26**	
481	**BRCA2 probe** 20550-L28144		**Exon 11**	
490 § »	**CHEK2 probe** 01772-L01336			**1100delC**
500 *	Reference probe 21229-L29604	10p11		

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

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
¤ Changed in version D1. The original 3-part probe was replaced by a 2-part probe to reduce sensitivity to DNA depurination.
§ Mutation-specific probe. This probe will only generate a signal when the *CHEK2* 1100delC mutation is present.
∞ Wild type sequence detected. A lowered probe signal can be due to a *BRCA2* exon 3 deletion or due to the presence of the c.156_157insAlu (Portuguese founder) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
¬ 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.
Ж A high signal of the 409 nt probe can be due to depurination of the sample DNA, e.g. due to insufficient buffer concentration in the DNA sample. When this occurs in reference samples, it can look like a decreased signal for this probe in the test samples. The 232 nt probe (01603-L13850) can show a similar trend, whereas probes 130 nt (00797-L00463), 149 nt (20546-L28140) and 166 nt (20603-L28261) will show the opposite trend. Please consult the Support section on www.mlpa.com for more information on depurination.

Table 2. P045-D1 probes arranged according to chromosomal location

Table 2a. *BRCA2*

Length (nt)	SALSA MLPA probe	BRCA2 exon[a]	Ligation site NM_000059.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*228-230 (Exon 2)*		
244 ¬	20548-L31554	Upstream	1665 nt before exon 1	AGAGAACAAGAA-ACATAAAGGTAT	1.7 kb
136	02283-L26707	Exon 1	0-1	CAGCGCGGGCTT-GTGGCGCGAGCT	0.2 kb
154	02285-L23744	Exon 1	23 nt after exon 1	TGGTAGTGGGTT-GGGACGAGCGCG	0.8 kb
172	02486-L23747	Exon 2	271-270 reverse	AGCGTGTCTTAA-AAATTTCAAAAA	2.7 kb
238 ∞	22219-L31553	Exon 3	381-382; WT at c.156_157insAlu	AAGAATCTGAAC-ATAAAAACAACA	0.1 kb
426	20631-L25993	Exon 3	472-473	AATAATATTCAA-AGAGCAAGGGCT	5.9 kb
202	01600-L23751	Exon 4	569-570	AATAGTAGACAT-AAAAGTCTTCGC	1.0 kb
321	09809-L28325	Exon 5	688-689	TGTAACACCACA-AAGAGATAAGTC	0.1 kb
355	04585-L23764	Exon 6	728-727 reverse	ACAAACTTTGGT-GTATGAAACAAA	0.3 kb
208	08265-L23752	Exon 7	812-813	ATGTCTTGGTCA-AGTTCTTTAGCT	2.9 kb
454	20632-L28323	Exon 8	893-892 reverse	GTAGTATCATGA-GGAAATACAGTT	1.5 kb
232	01603-L13850	Exon 9	1001-1002	AACACAAATCAA-AGAGAAGCTGCA	1.6 kb
250	01604-L23754	Exon 10	1374-1375	GAAGTGACAAAA-TCTCCAAGGAAG	0.5 kb
220	18388-L23375	Exon 10	1914-1913 reverse	GGTGGCTGGCCA-GCTTCCATTATC	0.2 kb
391	20543-L28130	Exon 10	2104-2105	AAATGCTTTTGA-AGCACCACTTAC	3.0 kb
265	20549-L28781	Exon 11	2244-2243 reverse	ACATGTTTCATT-TCTAGAACATTT	1.0 kb
142	18385-L23778	Exon 11	3249-3250	GTTTTGGAGGTA-GCTTCAGAACAG	0.7 kb
166	20603-L28261	Exon 11	3955-3954 reverse	TATTCTCAATAT-CACTAAACAGTT	1.3 kb
190	18387-L24251	Exon 11	5219-5218 reverse	GCTGAATTTTCA-ATGACTGAATAA	1.1 kb
481	20550-L28144	Exon 11	6273-6274	CCAAAGTATTGT-TTAAAAGTAACG	0.7 kb
283	01606-L23757	Exon 11	6992-6993	TCTCTTTTTACA-TGTCCCGAAAAT	3.5 kb
337	20628-L28320	Exon 12	7154-7155	GCTTCAAAAAGC-ACTCCAGATGGT	2.2 kb
313	02280-L28326	Exon 13	7216-7215 reverse	GTACACAGGTAA-TCGGCTCTAAAG	8.2 kb
160	09297-L28129	Exon 14	7394-7395	TCTGCTACAAGA-AATGAAAAAATG	1.5 kb
418	20630-L28322	Exon 15	7762-7763	CAGTCTGTATCT-TGCAAAAACATC	1.3 kb
346	01611-L23763	Exon 16	7975-7976	ACAGTTGGCTGA-TGGTGGATGGCT	4.8 kb
364	02281-L23765	Exon 17	8158-8157 reverse	TTAGGCATCTAT-TAGCAAATTCCT	0.8 kb
291	20676-L28319	Exon 18	8482-8483	TCAGAAGATTAT-TCTTCATGGAGC	7.0 kb
149	20546-L28140	Exon 19	8618-8619	TTCTTTCCTGAC-CCTAGACCTTTT	0.5 kb
400	08266-L23768	Exon 20	8743-8744	ATCTGGATTATA-CATATTTCGCAA	5.7 kb
373	20629-L28321	Exon 21	8909-8910	ACAAGACAGCAA-GTTCGTGCTTTG	2.7 kb
184	20625-L28317	Exon 22	9100-9101	TGCTGAACAAAA-GGAACAAGGTTT	0.3 kb
196	09812-L23750	Exon 23	9214-9215	ATCATCAGATTT-ATATTCTCTGTT	0.3 kb
445	08267-L23772	Exon 24	9455-9454 reverse	GAAACGACAAAT-CCTATTAGGTCC	14.8 kb
226	20626-L28778	Exon 25	9706-9707	AGAGACATTCAA-CAAAATGAAAAA	2.0 kb
472	11984-L23775	Exon 26	9786-9787	TACTGCATGCAA-ATGATCCCAAGT	1.3 kb
295	20541-L28782	Exon 27	9988-9989	AAAGTCTTGTAA-AGGGGAGAAAGA	0.4 kb
328	19699-L28324	Exon 27	10375-10376	TCTCAGACTGAA-ACGACGTTGTAC	0.8 kb
275	18389-L24255	Exon 27	11139-11138 reverse	GAAACACCACTC-TTCATATTCATC	7.9 kb
		stop codon	*10482-10484 (Exon 27)*		
462 ¬	18948-L01619	N4BP2L1 (CG018) gene		CATTATTATTGA-TAATACCAACCT	

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
271 »	20724-L29194	Exon 1	3-4	TTTAGCGCCACT-CTGCTGGCTGAG	41.9 kb
409 » Ж	02579-L23769	Exon 9	994-995	CTGTTTGACAAA-GTGGTGGGGAAT	4.0 kb
490 » §	01772-L01336	Exon 11	1159-1157 reverse; 1100delC mutation	TGCCCAAAATCA-TAATCTAAAATT	

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

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

§ Mutation-specific probe. This probe will only generate a signal when the *CHEK2* 1100delC mutation is present.
∞ Wild type sequence detected. A lowered probe signal can be due to a *BRCA2* exon 3 deletion or due to the presence of the c.156_157insAlu (Portuguese founder) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.
¬ 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.
Ж A high signal of the 409 nt probe can be due to depurination of the sample DNA, e.g. due to insufficient buffer concentration in the DNA sample. When this occurs in reference samples, it can look like a decreased signal for this probe in the test samples. The 232 nt probe (01603-L13850) can show a similar trend, whereas probes 130 nt (00797-L00463), 149 nt (20546-L28140) and 166 nt (20603-L28261) will show the opposite trend. Please consult the Support section on www.mlpa.com for more information on depurination.

Related SALSA MLPA probemixes

P077 BRCA2 Confirmation:	Results obtained with P045/P090 can be confirmed with this probemix.
P090 BRCA2:	BRCA2 probes identical to P045, but does not contain CHEK2 probes.
P190 CHEK2:	Breast cancer susceptibility, genes included: *CHEK2*, *ATM*, *TP53*.
P002/P087 BRCA1:	Hereditary breast and ovarian cancer, screening *BRCA1*.
P239 BRCA1 region:	Characterisation of deletions/duplications upstream and downstream of *BRCA1*.
P056 TP53:	Mutations in *TP53* cause Li-Fraumeni syndrome.
P260 PALB2-RAD50-RAD51C-RAD51D:	Probes for the *PALB2*, *RAD51C*, *RAD51D* and *RAD50* genes, which have been linked to breast and/or ovarian cancer.
P041/P042 ATM:	Mutations in *ATM* have been linked to a higher risk of breast cancer.

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P045 Product history	
Version	*Modification*
D1	The BRCA2 exon 3/ c.156_157insALU probe has been changed from a 3-part to a 2-part probe in order to reduce its sensitivity to sample DNA depurination. One probe has a small change in length, but not in sequence detected. One reference probe has been replaced.
C1	The probes for the *BRCA2* upstream region and exons 8, 11, 12, 19 and 27 have been replaced, and extra probes have been added for longer exons. A probe detecting the *BRCA2* c.156_157insAlu mutation has been included. The BRCA2 exon 3 probe that detects the c.504del5068insCCAT mutation has been removed. For *CHEK2,* the exon 1 probe has been replaced. In addition, most reference probes have been replaced and the lengths of most target probes have been adjusted.
B3	The 88 and 96 nt DNA denaturation control fragments (QDX2) have been replaced.

B2	Four reference probes have been replaced and extra control fragments at 100 and 105 nt (X, Y chromosome specific) have been included.
B1	Three new probes for BRCA2 have been added and seven probes have been replaced as compared to previous versions of this probe set. In addition, two DNA denaturation control probes at 88 and 96 nt are now also included in P045B.
A0	Two extra BRCA2 probes (exons 6 and 26) and a new exon 25 probe has been added as compared to the previous lots 0204, 0804 & 0105.
A	First release.

Implemented changes in the product description

Version D1-02 – 31 March 2020 (02P)
- The removal of the BRCA2 exon 3/ c.504del5068insCCAT probe in product version C1 was added to the P045 product history.
- Costa Rica was added as country with IVD status.
- Various minor textual or layout changes.

Version D1-01 – 28 May 2019 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Intended use has been adjusted to include FA type D1.
- Information about FA type D1 was added to the clinical background section and performance characteristics section.
- Interpretation of results section concerning homozygous deletions (copy number 0) was updated.
- Warning was added for probe 02579-L23769 in Table 1 and 2b.
- Various minor textual or layout changes.
- Limitation on risk of positive results due to founder mutations was added.
- Reference section of probemixes using P045 updated.

Version C1-04 – 05 October 2018 (04)
- Product description restructured and adapted to a new template.
- Various minor textual or layout changes.
- Updated positive sample section with *CHEK2* 1100delC positive sample.
- Wording for *CHEK2* 1100delC note was adjusted.
- 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 P190 probemix.
- Updated the related probemixes section.
- References using probemix P045 were updated.
- Countries where product has IVD status was updated.

Version C1-03 – 26 September 2017(03)
- Information concerning P077 BRCA2 Confirmation was adjusted (Intended use, Table overview BRCA2 probemixes, Table 1 and 2 and confirmation of results section) due to an update of the P077 probemix.
- Intended use was adjusted to clarify the usage of the product.
- Information on positive sample from the Coriell Institute was added.
- Minor textual changes.

Version C1-02 – 08 June 2017 (03)
- Information concerning BRCA2 probe 18503-SP0658-L28779 (Table 1 and 2 and confirmation of results section) adjusted.
- Chromosome position updated according to current NCBI information.

Version C1-01 – 05 December 2016 (03)
- Product description restructured and adapted to a new template.

Version 34 – 21 July 2016 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- Various textual changes.

Version 33 – 09 September 2015 (55)

- Product description adapted to a new lot (lot number added, new pictures included).
- Manufacturer's address adjusted.

Version 32 – 27 May 2015 (54)

- Information about the BRCA2 added in the first paragraph and references updated.

Version 31 – 18 February 2015 (54)

Exon numbering of the CHEK2 gene changed in Table 1 and Table 2b.

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