Product Description SALSA® MLPA® probemix P090-B1 BRCA2

To be used with the MLPA General Protocol.

Version B1. As compared to version A4, 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 wild type sequence for the c.156_157insAlu mutation has been included. Most reference probes have been replaced and one has been added. In addition, the lengths of most target probes have been adjusted. For complete product history see page 8.

Catalogue numbers:
- **P090-025R:** SALSA MLPA probemix P090 BRCA2, 25 reactions.
- **P090-050R:** SALSA MLPA probemix P090 BRCA2, 50 reactions.
- **P090-100R:** SALSA MLPA probemix P090 BRCA2, 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).

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 P090 BRCA2 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). This product 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 and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials. Deletions or duplications detected with the P090 BRCA2 probemix must be verified by using the SALSA MLPA probemix P077 BRCA2 Confirmation or a different technique. 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 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: 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 of 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). More information is available at http://www.ncbi.nlm.nih.gov/books/NBK1247/.

Deletions or duplications are more frequent for *BRCA1* than for *BRCA2.* 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).

Gene structure: The *BRCA2* gene spans 84 kilobases (kb) on chromosome 13q13.1. The *BRCA2* LRG_293 is available at www.lrg-sequence.org and is identical to GenBank NG_012772.3.

Transcript variants: 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.

Exon numbering: The exon numbering used in this P090-B1 BRCA2 product description and in the P090-B1 BRCA2 Coffalyser sheet is identical to the exon numbering in the LRG_293 and NCBI NG_012772.3 reference sequence. Exon numbering used here may differ from literature. Please notify us of any mistakes.

P090-B1 probemix content: This SALSA MLPA probemix P090-B1 BRCA2 contains 51 MLPA probes with amplification products between 130 and 499 nt (Table 1) including 40 probes for the *BRCA2* gene region (Table 2) and 11 reference probes that detect sequences outside this region. The identity of the genes detected by the reference probes is 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 the sequence upstream and a probe for the sequence downstream of *BRCA2*.

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity Fragments (Q-fragments), three DNA Denaturation Fragments (D-fragments), and one chromosome X and one chromosome Y-specific fragment (Table 1). The Q-fragments are only visible when less than 100 ng sample DNA is used. Low signal of the 88 and 96 nt fragment indicates incomplete DNA denaturation. More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol.

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: Human DNA extracted 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: Reference DNA samples should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method as the patient samples. 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: 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 several probes when using the following probemixes: P002 and P087 BRCA1; P045, P090 and P077 BRCA2. 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 SD024 is for research use only (RUO).**

Sample ID numbers NA03330 and NA02718 from the Coriell Institute (https://catalog.coriell.org/) can be used as positive control samples to detect a whole gene duplication or whole gene deletion of *BRCA2*, respectively. NA03330 contains DNA with a trisomy of chromosome 13, and NA02718 contains DNA with a partial deletion of chromosome 13q.

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/). The analytical sensitivity and specificity for the detection of deletions or duplications in the *BRCA2* gene is very high and can be considered >99% (based on a 2011-2014 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 Coffalyser sheet. For both, the latest version should be used which are 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, see the Coffalyser.Net Manual.

Interpretation of results: The expected results for *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 cannot be expected since such a mutation is embryonically lethal.

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:

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 within a CpG island in or near the *BRCA2* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases.
- False positive duplication results: Contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to false positive duplication results (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 are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *BRCA2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P090 BRCA2.
- 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. Deletions or duplications detected by only a single probe always require confirmation by another method.

Confirmation of results: Deletions or duplications obtained with the P090 BRCA2 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 P090 BRCA2 or probemix P045 BRCA2/CHEK2. The c.156_157insAlu mutation must be verified with another method, such as nested PCR (Machado et al. 2007).

BRCA1/2 mutation database: 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. 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 P090-B1 BRCA2

Length (nt)	SALSA MLPA probe	Chromosomal position[(a)] reference	*BRCA2*
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)		
88-92-96	D-fragments (Low signal of 88 or 96 fragment indicates incomplete denaturation)		
100	X-fragment (X chromosome specific)		
105	Y-fragment (Y chromosome specific)		
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 Ж ∞	**BRCA2 probe** 18503-SP0658-L28779 *		**Exon 3/ c.156_157insAlu**
244	**BRCA2 probe** 20548-L28142 *		**Upstream**
250	**BRCA2 probe** 01604-L23754 ¥		**Exon 10**
257	Reference probe 02469-L28780 *	15q21	
265	**BRCA2 probe** 20549-L28781 *		**Exon 11**
269	Reference probe 03075-L20665 *	5p15	
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	Reference probe 15392-L17223 *	3p22	
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	Reference probe 12461-L21828 *	22q12	
499	Reference probe 14882-L21050 *	14q11	

(a) The exon numbering used in this P090-B1 BRCA2 product description and in the P090-B1 BRCA2 Coffalyser sheet is identical to the exon numbering in the LRG_293 and NCBI NG_012772.3 reference sequence.

* New in version B1 (from lot B1-1115 onwards).
¥ Changed in version B1 (from lot B1-1115 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. When this occurs in reference samples, it can look like an increased signal in the test samples.
∞ Wild type sequence detected. The presence of the c.156_157insAlu (Portuguese founder) mutation will result in a decreased probe 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.

Table 2. BRCA2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*BRCA2* Exon[a]	Ligation site[b] NM_000059.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
244	20548-L28142	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
		start codon	*228-230 (exon 2)*		
172	02486-L23747	Exon 2	271-270 reverse	AGCGTGTCTTAA-AAATTTCAAAAA	2.6 kb
238 Ж ∞	18503-SP0658-L28779	Exon 3	349-350 and 385-386; WT at c.156_157insAlu	AGAAGCTCCACC-36nt spanning oligo-AAACAACAATTA	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
		stop codon	*10482-10484 (exon 27)*		
275	18389-L24255	Exon 27	11139-11138 reverse	GAAACACCACTC-TTCATATTCATC	7.9 kb
462 ¬	18948-L01619	N4BP2L1 (CG018) gene		CATTATTATTGA-TAATACCAACCT	

(a) The exon numbering used in this P090-B1 BRCA2 product description and in the P090-B1 BRCA2 Coffalyser sheet is identical to the exon numbering in the LRG_293 and NCBI NG_012772.3 reference sequence.

(b) Ligation sites of the P090 BRCA2 MLPA probes are indicated according to RefSeq sequence NM_000059.3 containing 27 exons.
(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

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. When this occurs in reference samples, it can look like an increased signal in the test samples.
∞ Wild type sequence detected. The presence of the c.156_157insAlu (Portuguese founder) mutation will result in a decreased probe 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.

Related SALSA MLPA probemixes

P077 BRCA2 Confirmation:	Results obtained with P045/P090 can be confirmed with this probemix.
P045 BRCA2/CHEK2:	BRCA2 probes identical to P090, but also contains CHEK2 probes.
P002/P087 BRCA1:	Hereditary breast and ovarian cancer, screening *BRCA1*.
P239 BRCA1 region:	Characterisation of deletions/duplications upstream and downstream of *BRCA1*.
P190 CHEK2:	Breast cancer susceptibility, genes included: *CHEK2*, *ATM*, *BRCA1*, *PTEN*, *TP53*.
P057 FANCD2/PALB2:	Mutations in *PALB2* have been linked to a higher risk of breast cancer.
P240 BRIP1/CHEK1:	Mutations in *BRIP1* have been linked to a higher risk of breast cancer.

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Selected publications using SALSA MLPA Probemix P090 BRCA2

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P090 Product history	
Version	*Modification*
B1	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 wild type sequence for the c.156_157insAlu mutation has been included. Most reference probes have been replaced and one has been added. In addition, the lengths of most target probes have been adjusted.
A4	The 88 and 96 nt DNA denaturation control fragments (QDX2) have been replaced.
A3	Four reference probes have been replaced and two BRCA2 probes have a small change in length.
A2	Two extra control fragments at 100 and 105 nt (X, Y chromosome specific) have been included. Probes at 137 nt and 148 nt have been slightly modified. No change in detected sequences.
A1	First release.

Implemented changes in the product description

Version B1-04 – 11 January 2019 (03)
- Product is now registered for IVD use in Colombia, Morocco and Israel.

Version B1-03 - 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 update of P077 probemix.
- Intended use was adjusted to clarify the usage of the product.
- Minor textual changes.
- Information on positive sample from the Coriell Institute was added.

Version B1-02 – 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 B1-01 – 05 December 2016 (03)
- Product description restructured and adapted to a new template.

Version 19 – 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 picture included).
- Various textual changes.

Version 18 – 09 September 2015 (55)
- Product description adapted to a new lot (lot number added, new picture included).
- Manufacturer's address adjusted.

Version 17 (53)
- Product description adapted to a new lot (lot number added, new picture included).

Version 16 (53)
- Product description adapted to a new lot (lot number added, new picture included).

Version 15 (52)
- Product description adapted to a new lot (lot number added, new picture included).

Version 14 (48)
- Textual change below Table 2.

Version 13 (48)
- Warning added in Table 1 and 2, 172 nt probe 08898-L09587.

Version 12 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 11 (48)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Various minor textual changes.

Version 10 (48)
- Ligation sites of the probes targeting the BRCA2 gene updated according to new version of the NM_reference sequence.
- Various minor textual changes.
- Remark on RefSeqGene standard added below Table 2.

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The product is for RUO in all other European countries.