Product Description SALSA® MLPA® Probemix P087-D1 BRCA1 Confirmation

To be used with the MLPA General Protocol.

Version D1. Fourteen BRCA1 probes have been replaced and two have been added. The BRCA2 probes have been removed. Several probes have a change in length but not in sequence. The majority of the reference probes have been replaced and two additional ones have been added. For complete product history see page 9.

Catalogue numbers:

- **P087-025R:** SALSA MLPA probemix P087 BRCA1 Confirmation, 25 reactions.
- **P087-050R:** SALSA MLPA probemix P087 BRCA1 Confirmation, 50 reactions.
- **P087-100R:** SALSA MLPA probemix P087 BRCA1 Confirmation, 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 P087 BRCA1 Confirmation is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the confirmation of deletions or duplications in the human *BRCA1* gene as initially observed using the SALSA MLPA probemix P002 BRCA1[2]. This assay is for use with human genomic DNA extracted from peripheral blood and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials. The 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).
[2]The P002 BRCA1 probemix should be used as first tier probemix, because its coverage of the *BRCA1* gene is more extensive.

Clinical background: Germline defects in the *BRCA1* gene are the most frequent cause of a hereditary predisposition to breast 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 and about 5 to 10% of all breast cancers. In addition, mutations in the *BRCA1* and *BRCA2* genes account for around 15% of ovarian cancers overall.
More information is available at http://www.ncbi.nlm.nih.gov/books/NBK1247/.

The great majority of germline defects in the *BRCA1* gene are point mutations that can be detected by sequence analysis. Deletions and duplications of complete exons in the *BRCA1* gene are the second most common cause of defects in the *BRCA1* gene. These copy number changes are usually missed by amplicon-based sequencing analysis (Sanger sequencing or Next Generation Sequencing), but can be detected by the MLPA technique and hence MLPA complements sequence analysis of the *BRCA1* gene. Large genomic rearrangements in *BRCA1* may account for up to one-third of all disease-causing mutations, dependent on the population (Hansen et al. 2009). For example in Italian hereditary breast and ovarian cancer (HBOC) families the prevalence is 23% (Montagna et al. 2003), in the Netherlands 27%-36% (Hogervorst et al. 2003; Petrij-Bosch et al. 1997), while in a Danish cohort of HBOC patients the prevalence was 3.8% (Thomassen et al. 2006).

Gene structure: The *BRCA1* gene spans ~80 kilobases (kb) on chromosome 17q21.31. A pseudogene with high sequence similarity to *BRCA1* exons 1a, 1b, and 2 is located 40 kb upstream of exon 1. All MLPA probes have been designed to detect only the *BRCA1* sequence and not the pseudogene. The *BRCA1* LRG_292 is available at www.lrg-sequence.org and is identical to GenBank NG_005905.2.

Transcript variants: Multiple transcript variants have been described: http://www.ncbi.nlm.nih.gov/gene/672. *BRCA1* transcript variant 1 (NM_007294.3, 7224 nt, coding sequence: 233-5824) represents the most abundant transcript, encoding the full-length protein. This sequence is a reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 2 and the stop codon is located in exon 24. *BRCA1* transcript variant 2 (NM_007300.3), variant 3 (NM_007297.3), variant 4 (NM_007298.3) and variant 5 (NM_007299.3) are rare variants that use alternative transcription start sites (exon 1b) and/or alternative in-frame splice sites in the coding sequence. The NM sequence used is from 05/2018, but can be changed (e.g. by NCBI) after the release of the product description.

Exon numbering: The exon numbering used in this P087-D1 BRCA1 Confirmation product description and in the P087-D1 BRCA1 Confirmation lot-specific Coffalyser.Net analysis sheet is the traditional exon numbering (exons 1a, 2, 3 and 5-24) wherein no exon 4 is present. **Please note that the *BRCA1* exon numbering in the *BRCA1* LRG sequence and in the NCBI NG_005905.2 reference sequence is different. In Table 1 and Table 2 the LRG exon numbering is indicated between brackets.**

Probemix content: This SALSA MLPA probemix P087 BRCA1 Confirmation contains 40 MLPA probes with amplification products between 130 nt and 463 nt (Table 1), including 28 probes for the *BRCA1* region (Table 2) and 12 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 major *BRCA1* transcript variant 1. Three probes are present for exon 11 and two probes for exon 1a and exon 13. To determine the extent of a deletion/duplication, one probe is present in the upstream region of *BRCA1* (0.9 kb before exon 1a).

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 human 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 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: Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and 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 NA18949 and NA14626 from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control sample to detect a deletion of *BRCA1* exons 15 and 16 or a duplication of exon 13, respectively. The quality of cell lines can change, therefore samples should be validated before use.

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 three 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 product is for research use only (RUO).**

Performance characteristics: The expected number of *BRCA1* deletions or duplications that can be detected with this MLPA probemix is between ~0.5 and 25% of all *BRCA1* pathogenic mutations, dependent on the population (Smith et al. 2011; Sluiter et al. 2011). The analytical sensitivity and specificity for the detection of deletions/duplications in the *BRCA1* gene in samples without point mutations in *BRCA1* (based on a 2010-2017 literature review), is very high and can be considered >99%.

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 *BRCA1* 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. Hogervorst et al. 2003). A homozygous deletion (copy number 0) of the *BRCA1* gene cannot be expected because such a deletion is associated with embryonic lethality.

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 or in or near the *BRCA1* gene. 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 are unlikely to have any relation to the condition tested for.

Notes *BRCA1* results**:**

- A hereditary predisposition to breast/ovarian cancer due to *BRCA1* gene defects is an autosomal dominant disorder. Inactivation of a single copy of the *BRCA1* gene is thus expected to be pathogenic.
- A heterozygous deletion of one or more *BRCA1* exons that are present in the major transcript variant NM_007294.3, including the non-coding exon 1a, is expected to result in a hereditary predisposition to breast cancer. The clinical significance of a deletion of only the probe upstream of exon 1 or only the exon 1a probe with the ligation site 67 nt from the exon, is not clear.
- Heterozygous deletions of the complete *BRCA1* gene have been described but are rare. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole gene. Whole gene deletions or duplications should therefore be confirmed by analysis of an *independent* DNA sample, to exclude false positive results.
- Deletions of exons 1a, 1b and 2 are relatively frequent (van den Ouweland et al. 2009), though lower probe signals for these exons should be treated with caution. The presence of salt in the DNA sample can lead to incomplete DNA denaturation, especially of the GC-rich region near exons 1a, 1b and 2.
- A duplication of an internal part of a gene usually results in a defective copy of that gene, as the duplicated sequence is typically located directly adjacent to the original sequence, resulting in a defective transcript. Duplication of the *complete BRCA1* gene is not expected to result in disease.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *BRCA1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P087 BRCA1 Confirmation.
- 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.
- Multiple (putative) founder mutations for *BRCA1* have been described, which can cause false positive results (see limitation above).

Confirmation of results: The ligation sites of all probes in the P087 BRCA1 Confirmation probemix have a distance of at least 20 nt from probe ligation sites of the P002 BRCA1 probemix. Detected copy number changes, which are different from those detected with the P002 BRCA1 probemix 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.

Rare cases are known in which divergent results are obtained between the MLPA first tier probemix and the confirmation probemix due to a deletion/duplication that has a breakpoint within an exon. Del Valle et al. 2011 reported a ~3.3 kb deletion in exon 20, which could be detected with the P002 BRCA1 probemix but not with the P087 BRCA1 Confirmation probemix. This deletion can be confirmed by PCR using standard *BRCA1* sequencing primers (exon 20 forward + exon 21 reverse). This should give a 6.5 kb fragment in normal individuals and a smaller fragment in case of a deletion starting in the middle of exon 20.

BRCA1 mutation database: http://research.nhgri.nih.gov/bic/; http://BRCA1.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 *BRCA1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P087-D1 BRCA1 Confirmation

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a] Reference	*BRCA1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L21056	5q31	
136 *	**BRCA1 probe** 21934-L30749		**Exon 11 (10)**
148 «	**BRCA1 probe** 02807-L01268		**Exon 1a**
155 *	**BRCA1 probe** 21935-L30750		**Exon 8 (7)**
160 *	Reference probe 10686-L30981	6p12	
169 ¥	**BRCA1 probe** 21955-L30982		**Exon 6 (5)**
175	**BRCA1 probe** 02811-L02240		**Exon 3**
185	**BRCA1 probe** 03398-L02254		**Exon 18 (17)**
193	Reference probe 03217-L02642	10q25	
202 ¥	**BRCA1 probe** 11457-L30983		**Exon 22 (21)**
209 ¥	**BRCA1 probe** 21956-L30984		**Exon 15 (14)**
219	**BRCA1 probe** 02814-L02243		**Exon 7 (6)**
226 *	Reference probe 10244-L30985	10p12	
233 *	**BRCA1 probe** 21936-L30751		**Exon 9 (8)**
243	Reference probe 19134-L25333	21q22	
252 *	**BRCA1 probe** 21937-L30986		**Exon 13 (12)**
259 * «	**BRCA1 probe** 21938-L30987		**Upstream**
267 *	**BRCA1 probe** 21939-L30988		**Exon 11 (10)**
274 *	Reference probe 12782-L30989	2q12	
283 *	**BRCA1 probe** 21940-L30990		**Exon 19 (18)**
290 ¥	**BRCA1 probe** 02819-L30991		**Exon 12 (11)**
296 *	Reference probe 18670-L30992	11p14	
310 *	**BRCA1 probe** 21941-L30756		**Exon 21 (20)**
319 *	Reference probe 15385-L17792	3p22	
328 ¥ «	**BRCA1 probe** 21957-L02239		**Exon 2**
337	**BRCA1 probe** 02822-L02251		**Exon 16 (15)**
347 *	**BRCA1 probe** 21942-L30757		**Exon 5 (4)**
355	**BRCA1 probe** 03822-L03285		**Exon 10 (9)**
364 *	Reference probe 07034-L30993	14q11	
373 *	**BRCA1 probe** 21943-L31019		**Exon 24 (23)**
382 *	**BRCA1 probe** 21944-L30759		**Exon 13 (12)**
391 *	**BRCA1 probe** 21945-L30760		**Exon 20 (19)**
400 *	Reference probe 17588-L30994	7q11	
409 *	**BRCA1 probe** 21946-L30761		**Exon 17 (16)**
418 *	**BRCA1 probe** 21947-L30995		**Exon 23 (22)**
427 *	Reference probe 08839-L22026	2p13	
436 ¥ «	**BRCA1 probe** 02100-L30996		**Exon 1a**
447 *	**BRCA1 probe** 21948-L30763		**Exon 11 (10)**
454 *	**BRCA1 probe** 21949-L30997		**Exon 14 (13)**
463 *	Reference probe 08738-L08749	9q21	

(a) The exon numbering used in this P087-D1 BRCA1 Confirmation product description and in the P087-D1 BRCA1 Confirmation lot-specific Coffalyser.Net analysis sheet is the traditional exon numbering (exons 1a, 2, 3 and 5-24) wherein no exon 4 is present. Please note that the *BRCA1* exon numbering in the *BRCA1* LRG sequence and in the NCBI NG_005905.2 reference sequence is different. In Table 1 and Table 2 the LRG exon numbering is indicated between brackets.

* New in version D1 (from lot D1-0318 onwards).
¥ Changed in version D1 (from lot D1-0318 onwards). Small change in length, no change in sequence detected.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

Table 2. BRCA1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	BRCA1 exon[a]	Ligation site[b] NM_007294.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*233-235 (Exon 2)*		
259 # «	21938-L30987	Upstream	0.9 kb upstream exon 1a	TTTCGCCAAGAA-GATTGGCTCTTA	0.8 kb
148 «	02807-L01268	Exon 1a	67 nt before exon 1a	TCATCCGGGGGC-AGACTGGGTGGC	0.1 kb
436 # «	02100-L30996	Exon 1a	67-68	CGTGGCAACGGA-AAAGCGCGGGAA	1.4 kb
328 # «	21957-L02239	Exon 2	299-298 reverse	GATGGGACACTC-TAAGATTTTCTG	8.3 kb
175	02811-L02240	Exon 3	361-360 reverse	ACTTACTTGCAA-AATATGTGGTCA	9.3 kb
347	21942-L30757	Exon 5 (4)	441-440 reverse	TTATATACCTTT-TGGTTATATCAT	1.6 kb
169	21955-L30982	Exon 6 (5)	516-517	TGCTTTTCAGCT-TGACACAGGTTT	0.7 kb
219	02814-L02243	Exon 7 (6)	611-610 reverse	GTAGCCCATACT-TTGGATGATAGA	4.4 kb
155	21935-L30750	Exon 8 (7)	751-750 reverse	GACGTCTTTTGA-GGTTGTATCCGC	2.5 kb
233	21936-L30751	Exon 9 (8)	781-780 reverse	GAAGAATCAGAT-CCTAAAAAATTT	1.4 kb
355	03822-L03285	Exon 10 (9)	884-883 reverse	TGCAGAATCCAA-ACTGATTTCATC	1.2 kb
447	21948-L30763	Exon 11 (10)	1088-1087 reverse	ACTGCTGTTCTC-ATGCTGTAATGA	1.9 kb
267	21939-L30988	Exon 11 (10)	2978-2977 reverse	AGGCTTGATATT-AGACTCATTCTT	1.2 kb
136	21934-L30749	Exon 11 (10)	4155-4154 reverse	TGTTTGTATTTG-CAGTCAAGTCTT	0.6 kb
290	02819-L30991	Exon 12 (11)	4400-4399 reverse	GGTTAAAATGTC-ACTCTGAGAGGA	8.4 kb
252	21937-L30986	Exon 13 (12)	4429-4428 reverse	TTATGTTGCATG-GTATCCCTCTGC	0.1 kb
382	21944-L30759	Exon 13 (12)	4494-4495	GTTAGAACAGCA-TGGGAGCCAGCC	6.0 kb
454	21949-L30997	Exon 14 (13)	4674-4673 reverse	TAGAACTATCTG-CAGACACCTCAA	2.1 kb
209	21956-L30984	Exon 15 (14)	4852-4853	CAACAGCTGGAA-GAGTCTGGGCCA	3.2 kb
337	02822-L02251	Exon 16 (15)	4996-4997	CCAGAGTCAGCT-CGTGTTGGCAAC	3.5 kb
409	21946-L30761	Exon 17 (16)	5281-5280 reverse	ACATGAGTAGTC-TCTTCAGTAATT	3.8 kb
185	03398-L02254	Exon 18 (17)	5371-5372	GGAAAATGGGTA-GTTAGCTATTTC	0.6 kb
283	21940-L30990	Exon 19 (18)	5424-5423 reverse	AAGTACTTACCT-CATTCAGCATTT	6.2 kb
391	21945-L30760	Exon 20 (19)	5427-5426 reverse	CTTCAAAATCAT-GCTGAAAGAAAC	6.1 kb
310	21941-L30756	Exon 21 (20)	5563-5562 reverse	AGGCTCTTACCT-GTGGGCATGTTG	1.9 kb
202	11457-L30983	Exon 22 (21)	5575-5574 reverse	TGTACCATCCAT-TCCAGTTGATCT	1.5 kb
418	21947-L30995	Exon 23 (22)	5692-5691 reverse	TTACCATGGAAG-CCATTGTCCTCT	1.9 kb
373	21943-L31019	Exon 24 (23)	5744-5743 reverse	ACTGTCCAACAC-CCACTCTCGGGT	
		stop codon	*5822-5824 (Exon 24)*		

(a) The exon numbering used in this P087-D1 BRCA1 Confirmation product description and in the P087-D1 BRCA1 Confirmation lot-specific Coffalyser.Net analysis sheet is the traditional exon numbering (exons 1a, 2, 3 and 5-24) wherein no exon 4 is present. Please note that the *BRCA1* exon numbering in the *BRCA1* LRG sequence and in the NCBI NG_005905.2 reference sequence is different. In Table 1 and Table 2 the LRG exon numbering is indicated between brackets.

(b) Ligation sites of the P087 BRCA1 Confirmation MLPA probes are indicated according to RefSeq sequence NM_007294.3 containing 23 exons. The NM sequence used is from 05/2018, but can be changed (e.g. by NCBI) after the release of the product description.

(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.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
This 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.

Related SALSA MLPA probemixes

- P002 BRCA1 — Hereditary breast and ovarian cancer, primary screening *BRCA1*.
- P239 BRCA1 region — Characterisation of *BRCA1* deletions/duplications extending upstream exon 1 or beyond exon 24.
- P045 BRCA2/CHEK2 — Hereditary breast and ovarian cancer, *BRCA2* and *CHEK2*.
- P090 BRCA2 — Identical to P045 BRCA2/CHEK2, but does not contain probes for *CHEK2*.
- P077 BRCA2 Confirmation — Results obtained with P045 or P090 can be confirmed with this probemix.
- P190 CHEK2 — Breast cancer susceptibility genes included: *CHEK2*, *ATM*, *TP53*.
- P260 PALB2-RAD50-RAD51C-RAD51D — Probes for the *PALB2*, *RAD51C*, *RAD51D* and *RAD50* genes, which have been linked to breast and/or ovarian cancer.
- P056 TP53 — Mutations in *TP53* have been linked to a higher risk of breast cancer.
- P041/P042 ATM — Mutations in *ATM* have been linked to a higher risk of breast cancer.
- P240 BRIP1/CHEK1 — Mutations in *BRIP1* have been linked to a higher risk of ovarian and breast cancer.

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Selected publications using SALSA MLPA Probemix P087 BRCA1 Confirmation

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P087 Product history	
Version	*Modification*
D1	Fourteen BRCA1 probes have been replaced and two have been added. The BRCA2 probes have been removed. Several probes have a change in length but not in sequence. The majority of the reference probes have been replaced and two additional ones have been added.
C1	Three reference probes have been replaced and three additional reference probes have been included. The 88 and 96 nt control fragments have been replaced.
B1	Four probes have been replaced. Two extra control fragments at 100 and 105 nt (X, Y specific) have been added.
A1	First release

Implemented changes in the product description

Version D1-01 – 04 June 2018 (04)
- Product name change from BRCA1 to BRCA1 Confirmation
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 24 – 15 January 2016 (55)
- Product description adapted to a new lot (lot number added, new picture included).

- Alternative exon numbers added between brackets in table 2.
- Several small textual changes.

Version 23 (53)
- Product description adapted to a new lot (lot number added, new picture included).

Version 22 (51)
- Product description adapted to a new version (lot number added, new picture included).

Version 21 (48)
- Warning added in Table 1, 130 nt probe 02269-L01761.

Version 20 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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*comprising EU (candidate) member states and
members of the European Free Trade Association (EFTA).
The product is for RUO in all other European countries.