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

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 10.

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 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.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 purpose
The SALSA MLPA Probemix P087 BRCA1 Confirmation is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *BRCA1* gene in genomic DNA isolated from human peripheral whole blood specimens. P087 BRCA1 Confirmation is intended to confirm a potential cause for and clinical diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome, as initially determined using the SALSA MLPA Probemix P002 BRCA1. The P002 BRCA1 probemix should be used as a first tier probemix, as it provides a more extensive coverage of the *BRCA1* gene.

Discordant results between the P087 BRCA1 Confirmation probemix and the P002 BRCA1 probemix should be investigated with a different technique.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[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).
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

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 (LGRs) in *BRCA1* may account for up to 25% of all disease-causing mutations, dependent on the population (Smith et al. 2011; Sluiter et al. 2011). For example in Italian 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. The *BRCA1* LRG_292 is available at www.lrg-sequence.org and is identical to GenBank NG_005905.2. A pseudogene with high sequence similarity to *BRCA1* exons 1a, 1b, and 2 is located 40 kb upstream of *BRCA1* exon 1. All MLPA probes have been designed to detect only the *BRCA1* sequence and not the pseudogene.

Transcript variants
For *BRCA1,* multiple transcript variants have been described: http://www.ncbi.nlm.nih.gov/gene/672. Transcript variant 1 is the most predominant and encodes the full-length protein (NM_007294.4, 7088 nt, coding sequence: 114-5705). *BRCA1* transcript variant 2 (NM_007300.4), variant 3 (NM_007297.4), variant 4 (NM_007298.3), and variant 5 (NM_007299.4) are rare variants that use alternative in-frame splice sites in the coding sequence.

Exon numbering
The *BRCA1* exon numbering used in this P087-D1 BRCA1 Confirmation product description is the traditional exon numbering (exons 1a, 1b, 2, 3 and 5-24), wherein no exon 4 is present. **Please note that the *BRCA1* exon numbering in the *BRCA1* LRG_292 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.** The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P087-D1 BRCA1 Confirmation contains 40 MLPA probes with amplification products between 130 and 463 nucleotides (nt). This includes 28 probes for the *BRCA1* gene region. 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* (1.0 kb before exon 1a). In addition, 12 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.mrcholland.com).

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

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.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
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 different 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 (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA18949 and NA14626 from the Coriell Institute have been tested with this P087-D1 probemix at MRC Holland and can be used as positive control samples to detect a deletion of *BRCA1* exons 15 and 16 or a duplication of *BRCA1* 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.mrcholland.com. **This product is for research use only (RUO).**

Performance characteristics
The expected number of *BRCA1* chromosomal rearrangements 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 2008-2020 literature review), is very high and can be considered >99%.

Analytical performance can be compromised by: SNVs or other polymorphisms 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.mrcholland.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 since such a deletion is associated with embryonic lethality.

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) 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 FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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 (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 are unlikely to have any relation to the condition tested for.
- 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: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

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_007294.4, 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 *BRCA1* exon 1a or only the *BRCA1* exon 1a probe with the ligation site 186 nt before 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 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 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 be pathogenic, as it does not cause recombination deficiency (Aref-Eshghi et al. 2020).

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *BRCA1* gene are small (point) mutations, none of which will 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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, such as long range PCR, qPCR, array CGH or Southern blotting. 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 *BRCA1* 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 *BRCA1* exon 20.

***BRCA1* mutation database**

https://databases.lovd.nl/shared/genes/BRCA1. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). 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 SNVs and unusual results (e.g., a duplication of *BRCA1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.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	5q	
136	**BRCA1 probe** 21934-L30749		**Exon 11 (10)**
148 « ๑	**BRCA1 probe** 02807-L01268		**Exon 1a**
155	**BRCA1 probe** 21935-L30750		**Exon 8 (7)**
160	Reference probe 10686-L30981	6p	
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	10q	
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	10p	
233	**BRCA1 probe** 21936-L30751		**Exon 9 (8)**
243	Reference probe 19134-L25333	21q	
252	**BRCA1 probe** 21937-L30986		**Exon 13 (12)**
259 « ๑	**BRCA1 probe** 21938-L30987		upstream
267	**BRCA1 probe** 21939-L30988		**Exon 11 (10)**
274	Reference probe 12782-L30989	2q	
283	**BRCA1 probe** 21940-L30990		**Exon 19 (18)**
290	**BRCA1 probe** 02819-L30991		**Exon 12 (11)**
296	Reference probe 18670-L30992	11p	
310	**BRCA1 probe** 21941-L30756		**Exon 21 (20)**
319	Reference probe 15385-L17792	3p	
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	14q	
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	7q	
409	**BRCA1 probe** 21946-L30761		**Exon 17 (16)**
418	**BRCA1 probe** 21947-L30995		**Exon 23 (22)**
427	Reference probe 08839-L22026	2p	
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	9q	

[a] See section Exon numbering on page 2 for more information.

« 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.
๑ The clinical significance of a deletion of only the probe upstream of *BRCA1* exon 1a or only the *BRCA1* exon 1a probe with the ligation site 186 nt before the exon is not clear.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Detected copy number changes, which are different from those detected with the P002 BRCA1 probemix require confirmation by another method.

Table 2. *BRCA1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*BRCA1* exon[a]	Ligation site NM_007294.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*114-116 (Exon 2)*		
259 « ๑	21938-L30987	Upstream	1.0 kb before Exon 1a	TTTCGCCAAGAA-GATTGGCTCTTA	0.8 kb
148 « ๑	02807-L01268	Exon 1a	186 nt before Exon 1a	TCATCCGGGGGC-AGACTGGGTGGC	0.1 kb
436 « #	02100-L30996	Exon 1a	52 nt before Exon 1a	CGTGGCAACGGA-AAAGCGCGGGAA	1.4 kb
328 « #	21957-L02239	Exon 2	180-179, reverse	GATGGGACACTC-TAAGATTTTCTG	8.3 kb
175	02811-L02240	Exon 3	242-241, reverse	ACTTACTTGCAA-AATATGTGGTCA	9.3 kb
347	21942-L30757	Exon 5 (4)	322-321, reverse	TTATATACCTTT-TGGTTATATCAT	1.6 kb
169	21955-L30982	Exon 6 (5)	397-398	TGCTTTTCAGCT-TGACACAGGTTT	0.7 kb
219	02814-L02243	Exon 7 (6)	492-491, reverse	GTAGCCCATACT-TTGGATGATAGA	4.4 kb
155	21935-L30750	Exon 8 (7)	632-631, reverse	GACGTCTTTTGA-GGTTGTATCCGC	2.5 kb
233	21936-L30751	Exon 9 (8)	662-661, reverse	GAAGAATCAGAT-CCTAAAAAATTT	1.4 kb
355	03822-L03285	Exon 10 (9)	765-764, reverse	TGCAGAATCCAA-ACTGATTTCATC	1.2 kb
447	21948-L30763	Exon 11 (10)	969-968, reverse	ACTGCTGTTCTC-ATGCTGTAATGA	1.9 kb
267	21939-L30988	Exon 11 (10)	2859-2858, reverse	AGGCTTGATATT-AGACTCATTCTT	1.2 kb
136	21934-L30749	Exon 11 (10)	4036-4035, reverse	TGTTTGTATTTG-CAGTCAAGTCTT	0.6 kb
290	02819-L30991	Exon 12 (11)	4281-4280, reverse	GGTTAAAATGTC-ACTCTGAGAGGA	8.4 kb
252	21937-L30986	Exon 13 (12)	4310-4309, reverse	TTATGTTGCATG-GTATCCCTCTGC	0.1 kb
382	21944-L30759	Exon 13 (12)	4375-4376	GTTAGAACAGCA-TGGGAGCCAGCC	6.0 kb
454	21949-L30997	Exon 14 (13)	4555-4554, reverse	TAGAACTATCTG-CAGACACCTCAA	2.1 kb
209	21956-L30984	Exon 15 (14)	4733-4734	CAACAGCTGGAA-GAGTCTGGGCCA	3.2 kb
337	02822-L02251	Exon 16 (15)	4877-4878	CCAGAGTCAGCT-CGTGTTGGCAAC	3.5 kb
409	21946-L30761	Exon 17 (16)	5162-5161, reverse	ACATGAGTAGTC-TCTTCAGTAATT	3.8 kb
185	03398-L02254	Exon 18 (17)	5252-5253	GGAAAATGGGTA-GTTAGCTATTTC	0.6 kb
283	21940-L30990	Exon 19 (18)	5305-5304, reverse	AAGTACTTACCT-CATTCAGCATTT	6.2 kb
391	21945-L30760	Exon 20 (19)	5308-5307, reverse	CTTCAAAATCAT-GCTGAAAGAAAC	6.1 kb
310	21941-L30756	Exon 21 (20)	5444-5443, reverse	AGGCTCTTACCT-GTGGGCATGTTG	1.9 kb
202	11457-L30983	Exon 22 (21)	5456-5455, reverse	TGTACCATCCAT-TCCAGTTGATCT	1.5 kb
418	21947-L30995	Exon 23 (22)	5573-5572, reverse	TTACCATGGAAG-CCATTGTCCTCT	1.9 kb
373	21943-L31019	Exon 24 (23)	5625-5624, reverse	ACTGTCCAACAC-CCACTCTCGGGT	
		stop codon	*5703-5705 (Exon 24)*		

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.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.
๑ The clinical significance of a deletion of only the probe upstream of *BRCA1* exon 1a or only the *BRCA1* exon 1a probe with the ligation site 186 nt before the exon is not clear.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Detected copy number changes, which are different from those detected with the P002 BRCA1 probemix require confirmation by another method.

Related SALSA MLPA probemixes

P002 BRCA1	Contains probes for the *BRCA1* gene. It should be used for primary screening of *BRCA1*.
P239 BRCA1 region	Contains probes for the *BRCA1* region. It can be used to characterise deletions/duplications extending upstream or downstream of *BRCA1*.
P045 BRCA2/CHEK2	Contains probes for the *BRCA2* and *CHEK2* genes, involved in breast and ovarian cancer.
P090 BRCA2	Contains the same probes for the *BRCA2* gene as P045.
P077 BRCA2 Confirmation	Contains probes for the *BRCA2* gene. It can be used to confirm the results obtained with P045 or P090.
P190 CHEK2	Contains probes for the *CHEK2*, *ATM* and *TP53* genes, involved in cancer.
P041/P042 ATM	Contain probes for the *ATM* gene, involved in breast cancer and Ataxia Telangiectasia.
P056 TP53	Contains probes for *TP53*, involved in Li-Fraumeni syndrome.
P240 BRIP1/CHEK1	Contains probes for the *BRIP1* and *CHEK1* genes, involved in breast and ovarian cancer.
P260 PALB2-RAD50-RAD51C-RAD51D	Contains probes for the *PALB2, RAD50, RAD51C* and *RAD51D* genes, involved in breast and ovarian cancer.

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- del Valle J et al. (2010). Identification and comprehensive characterization of large genomic rearrangements in the BRCA1 and BRCA2 genes. *Breast Cancer Res Treat.* 122:733-43.
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- Janavičius R et al. (2014). Comprehensive BRCA1 and BRCA2 mutational profile in Lithuania. *Cancer Genet.* 207:195-205.
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P087 product history	
Version	*Modification*
D1	Fourteen BRCA1 probes have been replaced and two have been added. Probes for *BRCA2* have been removed. Several probes have a change in length but not in sequence detected. The majority of reference probes has been replaced and two additional reference probes have been added.
C1	Three reference probes have been replaced and three additional reference probes have been included. The 88 and 96 nt DNA denaturation control fragments have been replaced (QDX2).
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 D1-05 – 7 June 2021 (04P) - Intended purpose updated. - Various minor textual changes. - Warning present in Table 2 for probe at 259 nt regarding one nucleotide difference was removed. - Warning added to Table 1 and 2 regarding the clinical significance of several probes. - Section Notes *BRCA1* results updated. - One reference added and one reference removed from the reference section. Version D1-04 – 15 February 2021 (04P) - Product description adapted to a new template. - Various minor textual changes. - Transcript variants section updated. - Link to *BRCA1* mutation database updated. - Ligation sites of the probes targeting the *BRCA1* gene updated according to new version of the NM_ reference sequence.

- List of selected publications using SALSA MLPA Probemix P087 BRCA1 Confirmation updated.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version D1-03 – 21 August 2020 (04)
- Product is now registered for IVD use in Costa Rica.

Version D1-02 – 27 April 2020 (04)
- Product is now registered for IVD use in Colombia and Israel.

Version D1-01 – 04 June 2018 (04)
- Product name changed 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.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

IVD	EUROPE* CE COLOMBIA COSTA RICA ISRAEL
RUO	ALL OTHER COUNTRIES

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