

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 assay² 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

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 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 diseases such as prostate and breast cancer among male relatives. Mutations in the *BRCA1* and *BRCA2* genes account for about 20-25% of hereditary breast cancers (Easton 1999) and about 5-10% of all breast cancers (Campeau et al. 2008). In addition, mutations in the *BRCA1* and *BRCA2* genes account for around 15% of ovarian cancers (Pal et al. 2005). Women with a germline *BRCA1* mutation have a 55-72% risk of developing breast cancer by age 70, while the risk of women in the general population is 12%. The lifetime risk of developing ovarian cancer in women with a germline *BRCA1* mutation is 39-44%, compared to 1-2% in the general population.

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 MLPA and hence MLPA complements sequence analysis of the *BRCA1* gene. CNVs in *BRCA1* account for 11-13% of all *BRCA1* pathogenic mutations, dependent on the population. Some populations have a higher prevalence of CNVs in HBOC patients. 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).

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

Gene structure

The *BRCA1* gene spans ~81 kilobases (kb) on chromosome 17q21.31. The *BRCA1* LRG_292 is available at <https://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 45 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).

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 or 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 (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Coriell Sample ID	Expected result
NA18949	Heterozygous deletion of BRCA1 exon 15 and 16
NA14626	Heterozygous duplication of BRCA1 exon 13

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* CNVs that can be detected with this MLPA probemix is between 11 and 13% of all *BRCA1* pathogenic mutations, dependent on the population. The analytical sensitivity and specificity for the detection of deletions or duplications in the *BRCA1* gene is very high and can be considered >99% (based on a 2010-2022 literature review).

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	$0.80 < \text{FR} < 1.20$
Homozygous deletion	$\text{FR} = 0$
Heterozygous deletion	$0.40 < \text{FR} < 0.65$
Heterozygous duplication	$1.30 < \text{FR} < 1.65$
Heterozygous triplication/homozygous duplication	$1.75 < \text{FR} < 2.15$
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 or flanking 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.

P087 specific notes:

- 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 or duplication of 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 ↵	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 ↵	Flanking 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.

↖ 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.

↵ The clinical significance of a deletion or duplication of 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
259 « ~	21938-L30987	Upstream		TTTCGCCAAGAA-GATTGGCTTAA	0.8 kb
148 « ☺	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
		start codon	114-116 (Exon 2)		
328 « #	21957-L02239	Exon 2	180-179, reverse	GATGGGACACTC-TAACGATTTCTG	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	GACGTCTTTGA-GGTTGTATCCGC	2.5 kb
233	21936-L30751	Exon 9 (8)	662-661, reverse	GAAGAACATCAGAT-CCTAAAAAAATT	1.4 kb
355	03822-L03285	Exon 10 (9)	765-764, reverse	TGCAGAACATCCAA-ACTGATTCATC	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	TGTTTGATTTG-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-TGGGAGGCCAGCC	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-GAGTCTGGCCA	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-CATTCAAGCATT	6.2 kb
391	21945-L30760	Exon 20 (19)	5308-5307, reverse	CTTCAAAATCAT-GCTGAAAGAAC	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-CCATTGTCTCT	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@mcholland.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.

~ 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.

☺ The clinical significance of a deletion or duplication of 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. Four probes in probemix P239 have the same ligation sites as probes present in probemix P002.
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 in probemix P045.
P077 BRCA2 Confirmation	Contains probes for the BRCA2 gene. It can be used to confirm the results obtained with probemix 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.

References

- Aref-Eshghi E et al. (2020). Genetic and epigenetic profiling of BRCA1/2 in ovarian tumors reveals additive diagnostic yield and evidence of a genomic BRCA1/2 DNA methylation signature. *J Hum Genet.* 65:865-73.
- Campeau PM et al. (2008). Hereditary breast cancer: new genetic developments, new therapeutic avenues. *Hum Genet.* 124:31-42.
- del Valle J et al. (2011). Identification of a new complex rearrangement affecting exon 20 of BRCA1. *Breast Cancer Res Treat.* 130:341-4.
- Easton DF (1999). How many more breast cancer predisposition genes are there? *Breast Cancer Res.* 1:14-17.
- Hogervorst FB et al. (2003). Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. *Cancer Res.* 63:1449-53.
- Montagna M et al. (2003). Genomic rearrangements account for more than one-third of the BRCA1 mutations in northern Italian breast/ovarian cancer families. *Hum Mol Genet.* 12:1055-61.
- Pal T et al. (2005). BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer.* 104:2807-16.
- Petrij-Bosch A et al. (1997). BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients. *Nat Genet.* 17:341-5.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Thomassen M et al. (2006). Low frequency of large genomic rearrangements of BRCA1 and BRCA2 in western Denmark. *Cancer Genet Cytogenet.* 168:168-71.
- van den Ouweleen AM et al. (2009). Deletion of exons 1a-2 of BRCA1: a rather frequent pathogenic abnormality. *Genet Test Mol Biomarkers.* 13:399-406.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in Confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P087 BRCA1 Confirmation

- Aktas D et al. (2010). Identification of point mutations and large rearrangements in the BRCA1 gene in 667 Turkish unselected ovarian cancer patients. *Gynecol Oncol.* 119:131-5.
- Bozsik A et al. (2020). Complex characterization of germline large genomic rearrangements of the BRCA1 and BRCA2 genes in high-risk breast cancer patients - Novel variants from a large national center. *Int J Mol Sci.* 21:4650.
- Cerutti R et al. (2010). Identification of the first case of germline duplication of BRCA1 exon 13 in an Italian family. *Fam Cancer.* 9:275-82.
- de Sabando AR et al. (2019). Genetic and clinical characterization of BRCA-associated hereditary breast and ovarian cancer in Navarra (Spain). *BMC Cancer.* 19:1145.
- 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.
- del Valle J et al. (2011). Identification of a new complex rearrangement affecting exon 20 of BRCA1. *Breast Cancer Res Treat.* 130:341-4.
- Herzog JS et al. (2021). Genetic epidemiology of BRCA1- and BRCA2-associated cancer across Latin America. *NPJ Breast Cancer.* 7:107.
- James PA et al. (2015). Large genomic rearrangements in the familial breast and ovarian cancer gene BRCA1 are associated with an increased frequency of high risk features. *Fam Cancer.* 14:287-95.
- Janavičius R et al. (2014). Comprehensive BRCA1 and BRCA2 mutational profile in Lithuania. *Cancer Genet.* 207:195-205.
- Maistro S et al. (2016). Germline mutations in BRCA1 and BRCA2 in epithelial ovarian cancer patients in Brazil. *BMC Cancer.* 16:934.
- Rashid MU et al. (2017). Contribution of BRCA1 large genomic rearrangements to early-onset and familial breast/ovarian cancer in Pakistan. *Breast Cancer Res Treat.* 161:191-201.
- Tedaldi G et al. (2020). Male Breast Cancer: Results of the Application of Multigene Panel Testing to an Italian Cohort of Patients. *Diagnostics (Basel).* 10:269.
- van der Merwe NC et al. (2020). The contribution of large genomic rearrangements in BRCA1 and BRCA2 to South African familial breast cancer. *BMC Cancer.* 20:391.

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 D1-06 – 01 December 2022 (04P)

- Sections Clinical background and Performance characteristics updated with new information.
- Clarified that flanking probes are unlikely to have any relation to the condition tested for in the section Interpretation of results.
- The 259 nt probe was denoted as flanking probe in Table 1, and a flanking probe remark was added to Table 1 and 2.
- Sections References and Selected publications curated.
- Minor textual and layout changes.

Version D1-05 – 07 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 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@mcholland.com (information & technical questions) order@mcholland.com (orders)
Phone	+31 888 657 200

IVD	EUROPE*  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.