

Product Description SALSA® MLPA® Probemix P090-C1 BRCA2

To be used with the MLPA General Protocol.

Version C1. As compared to version B1, the exon 3/ c.156_157insALU probe has been changed from a 3-part to a 2-part probe in order to reduce its sensitivity to sample DNA depurination. One probe has a small change in length, but not in sequence detected. One reference probe has been removed. For complete product history see page 9.

Catalogue numbers:

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

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

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

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

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

Intended use: The SALSA MLPA probemix P090 BRCA2 is an in vitro diagnostic (IVD)¹ or a research use only (RUO) assay for the detection of deletions or duplications in the human *BRCA2* gene and the presence of the c.156_157insAlu mutation, in order to confirm a potential cause and clinical diagnosis for hereditary breast and ovarian cancer (HBOC) or in rare cases Fanconi Anemia type D1. This product can also be used for molecular genetic testing of at-risk family members.

This assay is for use with human DNA extracted from peripheral blood and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials. Deletions or duplications detected with the P090 BRCA2 probemix must be verified by using the SALSA MLPA probemix P077 BRCA2 Confirmation or a different technique. Most defects in the *BRCA2* gene are point mutations, the majority of which will not be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of the *BRCA2* gene. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

¹Please note that this probemix is for in vitro diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

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

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

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

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

Gene structure: The *BRCA2* gene spans 84 kilobases (kb) on chromosome 13q13.1 and contains 27 exons. The *BRCA2* LRG_293 is available at www.lrg-sequence.org and is identical to GenBank NG_012772.3.

Transcript variants: One transcript variant has been described encoding the full length protein (NM_000059.3; 11386 nt; coding sequence 228-10484; <http://www.ncbi.nlm.nih.gov/gene/675>). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2 and the stop codon is located in exon 27.

Exon numbering: The exon numbering used in this P090-C1 BRCA2 product description is the exon numbering from the RefSeq transcript NM_000059.3, which is identical to the LRG_293 sequence. The exon numbering and NM_ sequence used have been retrieved on 01/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: This SALSA MLPA probemix P090-C1 BRCA2 contains 50 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 40 probes for the *BRCA2* gene region. In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

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

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 and 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 peripheral blood, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥ 3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of hereditary predisposition to cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (<https://catalog.coriell.org>) and the Leibniz Institute DSMZ (<https://www.dsmz.de/home.html>) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID numbers NA03330 and NA02718 from the Coriell Institute have been tested with this P090-C1 probemix at MRC-Holland and can be used as positive control samples. NA03330 contains DNA with a trisomy of chromosome 13, which includes a whole gene duplication of *BRCA2*, whereas NA02718 contains DNA with a partial deletion of chromosome 13q resulting in a whole gene deletion of *BRCA2*. 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 more probes when using the following probemixes: P045, P090 and P077 for *BRCA2* and P002 and P087 for *BRCA1*. The SD024 Artificial Duplication DNA is a mixture of human female genomic DNA and a titrated amount of plasmid containing selected probe target sequences. For further details, please consult the SD024 Artificial Duplication DNA product description, available online: www.mlpa.com. **This product is for research use only (RUO).**

Performance characteristics: The frequency of *BRCA2* deletions or duplications in hereditary breast and ovarian cancer families is $\sim 1\%$, dependent on the population (Walsh et al. 2006, <http://www.ncbi.nlm.nih.gov/books/NBK1247/>). No deletions or duplications in *BRCA2* have been described for FA type D1. The analytical sensitivity and specificity for the detection of deletions or duplications in the *BRCA2* gene in samples without point mutations in *BRCA2*, is very high and can be considered $>99\%$ (based on a 2011-2018 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary

electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

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

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

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

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

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *BRCA2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P090 BRCA2.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- Several (putative) founder mutations for *BRCA2* have been described, which can cause false positive results (see limitation above). This includes the *BRCA2* 999del5 (rs80359671) Finnish/Icelandic founder mutation (Hartikainen et al. 2007) in the BRCA2 exon 9 probe.

Confirmation of results: Deletions or duplications obtained with the P090 BRCA2 probemix must be verified by using the SALSA MLPA probemix P077 BRCA2 Confirmation or a different technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH. All probes included in SALSA MLPA probemix P077 BRCA2 Confirmation are different from those in probemix P090 BRCA2 or probemix P045 BRCA2/CHEK2. The c.156_157insAlu mutation must be verified with another method, such as nested PCR (Machado et al. 2007).

BRCA1/2 mutation databases: <http://research.nhgri.nih.gov/bic/>; <http://BRCA1.lovd.nl>; <http://BRCA2.lovd.nl>. We strongly encourage users to deposit positive results in the Breast Cancer Mutation Databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on <http://varnomen.hgvs.org/>.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *BRCA2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P090-C1 BRCA2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a	
		Reference	BRCA2
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136	BRCA2 probe 02283-L26707		Exon 1
142	BRCA2 probe 18385-L23778		Exon 11
149	BRCA2 probe 20546-L28140		Exon 19
154	BRCA2 probe 02285-L23744		Exon 1
160	BRCA2 probe 09297-L28129		Exon 14
166	BRCA2 probe 20603-L28261		Exon 11
172	BRCA2 probe 02486-L23747		Exon 2
178	Reference probe 04532-L03921	2q24	
184	BRCA2 probe 20625-L28317		Exon 22
190	BRCA2 probe 18387-L24251		Exon 11
196	BRCA2 probe 09812-L23750		Exon 23
202	BRCA2 probe 01600-L23751		Exon 4
208	BRCA2 probe 08265-L23752		Exon 7
214	Reference probe 11996-L12824	6q25	
220	BRCA2 probe 18388-L23375		Exon 10
226	BRCA2 probe 20626-L28778		Exon 25
232	BRCA2 probe 01603-L13850		Exon 9
238 ∞	BRCA2 probe 22219-L31553		Exon 3/ c.156_157insAlu
244 †	BRCA2 probe 20548-L31554		Upstream
250	BRCA2 probe 01604-L23754		Exon 10
257	Reference probe 02469-L28780	15q21	
265	BRCA2 probe 20549-L28781		Exon 11
269	Reference probe 03075-L20665	5p15	
275	BRCA2 probe 18389-L24255		Exon 27
283	BRCA2 probe 01606-L23757		Exon 11
291	BRCA2 probe 20676-L28319		Exon 18
295	BRCA2 probe 20541-L28782		Exon 27
304	Reference probe 11441-L28327	1q41	
313	BRCA2 probe 02280-L28326		Exon 13
321	BRCA2 probe 09809-L28325		Exon 5
328	BRCA2 probe 19699-L28324		Exon 27
337	BRCA2 probe 20628-L28320		Exon 12
346	BRCA2 probe 01611-L23763		Exon 16
355	BRCA2 probe 04585-L23764		Exon 6
364	BRCA2 probe 02281-L23765		Exon 17
373	BRCA2 probe 20629-L28321		Exon 21
382	Reference probe 13329-L14755	18q21	
391	BRCA2 probe 20543-L28130		Exon 10
400	BRCA2 probe 08266-L23768		Exon 20
409	Reference probe 15392-L17223	3p22	
418	BRCA2 probe 20630-L28322		Exon 15
426	BRCA2 probe 20631-L25993		Exon 3
436	Reference probe 07975-L07756	17q21	
445	BRCA2 probe 08267-L23772		Exon 24
454	BRCA2 probe 20632-L28323		Exon 8
462 †	N4BP2L1 probe 18948-L01619		Downstream
472	BRCA2 probe 11984-L23775		Exon 26
481	BRCA2 probe 20550-L28144		Exon 11
490	Reference probe 12461-L21828	22q12	

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

† Changed in version C1. Minor alteration, no change in sequence detected.

× Changed in version C1. The original 3-part probe was replaced by a 2-part probe to reduce sensitivity to DNA depurination.

∞ Wild type sequence detected. A lowered probe signal can be due to a *BRCA2* exon 3 deletion or due to the presence of the c.156_157insAlu (Portuguese founder) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

↪ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Table 2. *BRCA2* probes arranged according to chromosomal location

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

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

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

∞ Wild type sequence detected. A lowered probe signal can be due to a *BRCA2* exon 3 deletion or due to the presence of the c.156_157insAlu (Portuguese founder) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

↪ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Related SALSA MLPA probemixes

P077 BRCA2 Confirmation:	Results obtained with P045/P090 can be confirmed with this probemix.
P045 BRCA2/CHEK2:	BRCA2 probes identical to P090, but also contains three CHEK2 probes.
P002/P087 BRCA1:	Hereditary breast and ovarian cancer, screening <i>BRCA1</i> .
P239 BRCA1 region:	Characterisation of deletions/duplications upstream and downstream of <i>BRCA1</i> .
P190 CHEK2:	Breast cancer susceptibility, genes included: <i>CHEK2</i> , <i>ATM</i> , <i>TP53</i> .
P056 TP53:	Mutations in <i>TP53</i> cause Li-Fraumeni syndrome.
P260 PALB2-RAD50- RAD51C-RAD51D:	Probes for the <i>PALB2</i> , <i>RAD51C</i> , <i>RAD51D</i> and <i>RAD50</i> genes, which have been linked to breast and/or ovarian cancer.
P041/P042 ATM:	Mutations in <i>ATM</i> have been linked to a higher risk of breast cancer.

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P090 Product history	
<i>Version</i>	<i>Modification</i>
C1	The exon 3/ c.156_157insAlu has been changed from a 3-part to a 2-part probe in order to reduce its sensitivity to sample DNA depurination. One reference probe has been removed and one probe has a small change in length but not in sequence detected.
B1	The probes for the <i>BRCA2</i> upstream region and exons 8, 11, 12, 19 and 27 have been replaced, and extra probes have been added for longer exons. A probe detecting the wild type sequence for the c.156_157insAlu mutation has been included. The exon 3 probe that detects the c.504del5068insCCAT mutation has been removed. Most reference probes have been replaced and one has been added. In addition, the lengths of most target probes have been adjusted.
A4	The 88 and 96 nt DNA denaturation control fragments (QDX2) have been replaced.
A3	Four reference probes have been replaced and two BRCA2 probes have a small change in length.
A2	Two extra control fragments at 100 and 105 nt (X, Y chromosome specific) have been included. Probes at 137 nt and 148 nt have been slightly modified. No change in detected sequences.
A1	First release.

Implemented changes in the product description
<p><i>Version C1-02 – 27 March 2020 (02P)</i></p> <ul style="list-style-type: none"> - The removal of the BRCA2 exon 3/ c.504del5068insCCAT probe in product version B1 was added to the P090 product history. - Product description adapted to a new template. - Various minor textual changes. <p><i>Version C1-01 – 29 March 2019 (04)</i></p> <ul style="list-style-type: none"> - 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). - Intended use has been adjusted to include FA type D1. - Information about FA type D1 was added to the clinical background section and performance

characteristics section.

- Interpretation of results section concerning homozygous deletions (copy number 0) was updated.
- Various minor textual or layout changes.
- Limitation on risk of positive results due to founder mutations was added.
- Reference section of probemixes using P090 updated.

Version B1-04 – 11 January 2019 (03)

- Product is now registered for IVD use in Colombia, Morocco and Israel.

Version B1-03 - 26 September 2017 (03)

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

Version B1-02 – 08 June 2017 (03)

- Information concerning BRCA2 probe 18503-SP0658-L28779 (Table 1 and 2 and confirmation of results section) adjusted.
- Chromosome position updated according to current NCBI information.

Version B1-01 – 05 December 2016 (03)

- Product description restructured and adapted to a new template.

Version 19 – 21 July 2016 (55)

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

Version 18 – 09 September 2015 (55)

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

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IVD	EUROPE*  COLOMBIA MOROCCO ISRAEL
RUO	ALL OTHER COUNTRIES

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