Product Description
SALSA® MLPA® Probemix
P260-C1 PALB2-RAD50-RAD51C-RAD51D

To be used with the MLPA General Protocol.

Version C1
For complete product history see page 11.

Catalogue numbers:
- **P260-025R:** SALSA MLPA Probemix P260 PALB2-RAD50-RAD51C-RAD51D, 25 reactions.
- **P260-050R:** SALSA MLPA Probemix P260 PALB2-RAD50-RAD51C-RAD51D, 50 reactions.
- **P260-100R:** SALSA MLPA Probemix P260 PALB2-RAD50-RAD51C-RAD51D, 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 P260 PALB2-RAD50-RAD51C-RAD51D is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplication in the human *PALB2*, *RAD51C*, *RAD51D* and *RAD50* genes in genomic DNA isolated from human peripheral whole blood specimens. P260 PALB2-RAD50-RAD51C-RAD51D is intended to confirm a potential cause for breast, ovarian and other cancer types in patients who are negative for *BRCA1* and *BRCA2* mutations. This product can also be used to determine increased cancer susceptibility in at-risk family members. Moreover, copy number variations in *PALB2* can also confirm a potential cause for and clinical diagnosis of autosomal recessive Fanconi Anemia type N.

Copy number variations (CNVs) detected with P260 PALB2-RAD50-RAD51C-RAD51D should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *PALB2*, *RAD50*, *RAD51C* and *RAD51D* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Please note that this probemix covers all exons of *PALB2*, *RAD51C* and *RAD51D*, but not of *RAD50*.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, 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
PALB2, *RAD50*, *RAD51C* and *RAD51D* all play a role in DNA damage repair and a defect in one of these genes can lead to increased risk of tumour formation. For breast cancer, autosomal dominant mutations in the genes *BRCA1* and *BRCA2* are the most frequent cause, followed by mutations in *PALB2*, *CHEK2* and *ATM*, though with a much lower frequency (Buys et al. 2017). Mutations in *PALB2* may also increase the risk of developing pancreatic cancer, although the evidence is limited (see Table A). Defects in both *PALB2* copies can result in Fanconi Anemia (FA) type N. 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 N comprises less than one percent of the cases. FA type N is associated with an unusually severe predisposition to paediatric malignancies (https://www.ncbi.nlm.nih.gov/books/NBK1401/).

Autosomal dominant mutations in *RAD51C* or *RAD51D* result in increased risk for cancer, in particular ovarian cancer, while evidence of an association between *RAD50* mutations and cancer risk is currently limited (see Table A).

Table A. Cancer risk associated with mutations in *PALB2*, *RAD51C*, *RAD51D* and *RAD50*.
(Adapted from the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, version 1.2022)

Gene	Cancer	Absolute risk	Strength of evidence
PALB2	Breast	41-60%	Strong (with overrepresentation of triple-negative disease)
	Epithelial Ovarian	3-5%	Strong
	Pancreatic	5-10%	Limited
RAD51C	Breast	15-40%	Strong for ER/PR-negative breast cancer
	Epithelial Ovarian	>10%	Strong
RAD51D	Breast	15-40%	Strong for ER/PR-negative breast cancer
	Epithelial Ovarian	>10%	Strong
RAD50	Breast and Epithelial Ovarian	Currently insufficient evidence of an association	Insufficient

Gene structure
The *PALB2* gene spans ~38 kilobases (kb) on chromosome 16p12.1 and has 13 exons. The *PALB2* LRG_308 is available at www.lrg-sequence.org and is identical to GenBank NG_007406.1.

The *RAD51C* gene spans ~42 kb on chromosome 17q22 and has 9 exons. The *RAD51C* LRG_314 is available and identical to GenBank NG_023199.1.

The *RAD51D* gene spans ~28 kb on chromosome 17q12 and has 10 exons. The *RAD51D* LRG_516 is available and identical to GenBank NG_031858.1.

The *RAD50* gene spans ~90 kb on chromosome 5q31.1 and has 25 exons. The *RAD50* LRG_312 is available and is identical to GenBank NG_021151.2.

Transcript variants
For *PALB2*, one transcript has been described encoding the full length protein (NM_024675.4; 4008 nt; coding sequence 154-3714; https://www.ncbi.nlm.nih.gov/gene/79728). This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 13.

For *RAD51C*, multiple transcript variants have been described. Transcript variant 1 (NM_058216.3; 2562 nt; coding sequence 43-1173; https://www.ncbi.nlm.nih.gov/gene/5889) is the longest transcript and encodes the longest isoform. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 9.

For *RAD51D*, multiple transcript variants have been described. Transcript variant 1 (NM_002878.4; 9966 nt; coding sequence 248-1234; https://www.ncbi.nlm.nih.gov/gene/5892) encodes isoform 1. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 10.

For *RAD50*, one transcript has been described encoding the full length protein (NM_005732.4; 8272 nt; coding sequence 349-4287; https://www.ncbi.nlm.nih.gov/gene/10111). This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 25.

Exon numbering

The exon numbering used in this P260-C1 PALB2-RAD50-RAD51C-RAD51D product description is the exon numbering from the LRG_308 sequence for *PALB2*, LRG_314 for *RAD51C*, LRG_516 for *RAD51D*, and NG_021151.2 for *RAD50*. 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 P260-C1 PALB2-RAD50-RAD51C-RAD51D contains 50 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 13 probes for the *PALB2* gene, nine probes for the *RAD51C* gene, ten probes for the *RAD51D* gene, and eight probes for the *RAD50* gene. At least one MLPA probe is present for each exon of *PALB2, RAD51C* and *RAD51D*. For *RAD50*, the eight probes are distributed evenly over the gene, including the first and last exon. 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.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 whole blood specimens, 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 NA08039, HG00634, HG03857 and NA14230, from the Coriell Institute have been tested with this P260-C1 probemix at MRC Holland and can be used as a positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Coriell Sample ID	Altered target genes in P260-C1	Expected result
NA08039	*PALB2*	complete heterozygous *PALB2* gene duplication
HG00634	*PALB2*	heterozygous duplication of *PALB2* exon 13
HG03857	*PALB2*	heterozygous deletion of *PALB2* exon 5-7
NA14230	*RAD50*	complete heterozygous deletion of *RAD50*

Performance characteristics
The frequency of deletions or duplications in *PALB2* in breast cancer as well as other cancer types is <1% (Blanco et al. 2012, Janatova et al. 2013, Susswein et al. 2016, Tung et al. 2015). For FA-type N, the frequency of copy number variations in *PALB2* is estimated to be <5% (https://www.ncbi.nlm.nih.gov/books/NBK1401/). To date, deletions of *PALB2* exon 1-10 (Xia et al. 2007) and exon 5-6 (Toksoy et al. 2020) have been reported in FA patients.

The frequency of deletions or duplications in *RAD51C* in ovarian cancer and other cancer types is <1% (Kraus et al. 2017, Schnurbein et al. 2013, Susswein et al. 2016).

The frequency of deletions or duplications in *RAD51D* in ovarian cancer and other cancer types is <1% (Susswein et al. 2016, ClinVar at NCBI: gene *RAD51D*).

The frequency of deletions or duplications in *RAD50* in breast cancer as well as other cancer types is <1% (ClinVar at NCBI: gene *RAD50*). Please note that only eight out of 25 *RAD50* exons are covered in this probemix, which means that not all deletions or duplications can be detected with this probemix.

The analytical sensitivity and specificity for the detection of deletions or duplications in these genes is very high and can be considered >99% (based on a 2013-2022 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 *PALB2, RAD51C, RAD51D* and *RAD50* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication) or 4 (heterozygous triplication/homozygous duplication). A homozygous deletion (copy number 0) of these genes is unlikely to be found in blood derived DNA, as such a deletion is expected to be embryonically lethal. 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. 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *PALB2*, *RAD51C*, *RAD51D* and *RAD50* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P260 PALB2-RAD50-RAD51C-RAD51D.
- Not all exons of *RAD50* are covered in this probemix. 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.

Confirmation of results

The SALSA MLPA Probemix P057 FANCD2-PALB2 can be used for confirmation of results for seven PALB2 probes present in P260 PALB2-RAD50-RAD51C-RAD51D. The other six PALB2 probes cannot be confirmed with the P057 FANCD2-PALB2 probemix, as their ligation site is similar or close to the probes in the P260 PALB2-RAD50-RAD51C-RAD51D probemix (see Tables 1 and 2b). Please note that P057 FANCD2-PALB2 is a research use only (RUO) assay.

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent 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.

Mutation databases

***PALB2*:** https://databases.lovd.nl/shared/genes/PALB2
***RAD51C*:** https://databases.lovd.nl/shared/genes/RAD51C
***RAD51D*:** https://databases.lovd.nl/shared/genes/RAD51D
***RAD50*:** https://databases.lovd.nl/shared/genes/RAD50
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 *PALB2* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P260-C1 PALB2-RAD50-RAD51C-RAD51D

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*RAD50*	*PALB2*	*RAD51C*	*RAD51D*
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 19616-L26704	4p				
136	**RAD51C probe** 20193-L27481				**Exon 2**	
142	**RAD51D probe** 20194-L27482					**Exon 10**
148	**PALB2 probe** 20195-L27483			**Exon 9**		
154	**RAD50 probe** 20196-L27484		**Exon 14**			
160	Reference probe 09787-L10202	15q				
166	**RAD51D probe** 20198-L27486					**Exon 6**
172 »	**PALB2 probe** 07501-L27938			**Exon 10**		
178	**RAD51D probe** 20199-L27487					**Exon 1**
184	**RAD51C probe** 16393-L27657				**Exon 3**	
190	Reference probe 08067-L19457	9p				
196	**RAD50 probe** 20200-L27488		**Exon 25**			
202	**RAD51C probe** 16395-L27659				**Exon 1**	
208	**RAD50 probe** 20201-L27489		**Exon 10**			
217 *	**RAD51D probe** 21961-L30775					**Exon 3**
222 *	Reference probe 05709-L15344	3q				
233 *	**RAD51C probe** 21962-L30776				**Exon 6**	
238	**RAD50 probe** 20204-L27492		**Exon 23**			
245 »	**PALB2 probe** 20205-L28341			**Exon 2**		
253	**RAD51C probe** 16399-L28036				**Exon 9**	
262	Reference probe 16433-L28037	18q				
267	**RAD51D probe** 20206-L28038					**Exon 9**
274 »	**PALB2 probe** 16391-L27658			**Exon 1**		
281	**RAD51D probe** 20207-L28039					**Exon 7**
288	**PALB2 probe** 20208-L28040			**Exon 4**		
293	Reference probe 15724-L27656	12q				
301	**RAD51C probe** 16400-L18815				**Exon 4**	
310 »	**PALB2 probe** 07502-L22722			**Exon 11**		
319 *	**RAD51D probe** 21963-L30777					**Exon 8**
329 *	**RAD51C probe** 21964-L30778				**Exon 8**	
336	**RAD50 probe** 08999-L19778		**Exon 4**			
344	Reference probe 12785-L27941	2q				
350 »	**PALB2 probe** 07504-L27660			**Exon 13**		
359	**RAD51C probe** 20139-L27378				**Exon 7**	
368	**RAD51D probe** 20210-L27498					**Exon 4**
375 »	**PALB2 probe** 07497-L19690			**Exon 6**		
388	**RAD51D probe** 20211-L27499					**Exon 2**
395	Reference probe 11901-L27676	6p				
406	**PALB2 probe** 20212-L27500			**Exon 3**		
414	**RAD50 probe** 20213-L27501		**Exon 2**			
423	**PALB2 probe** 20214-L27943			**Exon 8**		
427	**RAD51C probe** 20215-L27503				**Exon 5**	
436	**RAD50 probe** 09002-L27944		**Exon 21**			
445	**RAD50 probe** 08998-L09098		**Exon 1**			
454 *	Reference probe 15515-L17370	7q				
463 *	**PALB2 probe** 21965-L30779			**Exon 12**		
477	**PALB2 probe** 20217-L27505			**Exon 5**		
485	**RAD51D probe** 20218-L27945					**Exon 5**
494	**PALB2 probe** 20219-L27946			**Exon 7**		
500	Reference probe 19555-L27674	2p				

[a] See section Exon numbering on page 3 for more information.

* New in version C1.
» Detects the same sequence as probes in SALSA MLPA Probemix P057-B2 FANCD2-PALB2.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P260-C1 probes arranged according to chromosomal location

Table 2a. *RAD50* at 5q31.1

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_005732.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*349-351 (Exon 1)*		
445	08998-L09098	Exon 1	398-399	GAGTTTTGGAAT-AGAGGACAAAGA	1.9 kb
414	20213-L27501	Exon 2	519-520	ACTGGAGATTTC-CCTCCTGGAACC	20.1 kb
336	08999-L19778	Exon 4	765-766	ATTGACCGAGAA-ATGATCAGTTCT	11.9 kb
208	20201-L27489	Exon 10	1833-1834	GAGAAAAACAGC-AATGTAGAAACC	12.2 kb
154	20196-L27484	Exon 14	2723-2724	CCTGACAGATGT-TACAATTATGGA	14.8 kb
436	09002-L27944	Exon 21	3653-3654	GGATGCTGAGGA-AAAGTATAGAGA	20.0 kb
238	20204-L27492	Exon 23	3959-3958, reverse	CTGCCTTTTGTC-CAGCACTGCATC	4.1 kb
196	20200-L27488	Exon 25	4261-4262	AATGCAGTGTTA-GCTCCCTGGGAT	
		stop codon	*4285-4287 (Exon 25)*		

Table 2b. *PALB2* at 16p12.1

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_024675.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*154-156 (Exon 1)*		
274 »	16391-L27658	Exon 1	133-134	ACGGCTGCTCTT-TTCGTTCTGTCG	3.1 kb
245 »	20205-L28341	Exon 2	239-240	AAGGGAATACAG-CAAGACACTAGC	0.3 kb
406	20212-L27500	Exon 3	5 nt after exon 3	CACTCAGGTAAA-TCTAGACCATTC	2.4 kb
288	20208-L28040	Exon 4	1296-1297	CCTAAGAGTCTT-AGCCTGGAAGCA	5.0 kb
477	20217-L27505	Exon 5	1917-1916, reverse	AATGGAGCCGTG-AAAGCATCATCA	1.2 kb
375 »	07497-L19690	Exon 6	2694-2695	CTTCCTGCTTCT-GATAGCATAAAC	2.9 kb
494	20219-L27946	Exon 7	2771-2772	CGTAGATGTGAG-TGCCATGTTTTG	2.3 kb
423	20214-L27943	Exon 8	2901-2902	TGTTTTATTTAG-GTTCCAGTATTA	1.1 kb
148	20195-L27483	Exon 9	3123-3124	CAACAAGTAGAA-GTCATGACGTTT	1.6 kb
172 »	07501-L27938	Exon 10	3200-3201	TATACTAACTTT-TGCTGAGGTCCA	7.4 kb
310 »	07502-L22722	Exon 11	3321-3320, reverse	CAGACTGAAGCT-TGGTAAGAATCA	6.1 kb
463	21965-L30779	Exon 12	3440-3439, reverse	TCGTCTTAGGGT-TAATCACAATGA	4.3 kb
350 »	07504-L27660	Exon 13	3543-3544	TGTGCAGCAGCA-ATCTTGACTTCT	
		stop codon	*3712-3714 (Exon 13)*		

Table 2c. *RAD51D* at 17q12

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_002878.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*248-250 (Exon 1)*		
178	20199-L27487	Exon 1	130-131	TCTCCTCCGGCA-GCCAGCGCGCCT	0.6 kb
388	20211-L27499	Exon 2	387-386, reverse	AGCTCACCTTGT-AAGACAAGCCAC	0.5 kb
217	21961-L30775	Exon 3	435-434 reverse	TCACGGGGAAAG-CCGAGAACTGAG	11.2 kb
368	20210-L27498	Exon 4	542-543	CTGGTCTCTATA-CTGGAGAAGTGA	0.3 kb
485	20218-L27945	Exon 5	5 nt before exon 5	AATGCCCCCACC-CCCAGGTATGTC	0.7 kb
166	20198-L27486	Exon 6	779-780	ACATCTTCCAGA-TGCTGGATGTGC	2.9 kb
281	20207-L28039	Exon 7	831-832	TCAGGTGACTGG-TTCTTCAGGAAC	0.3 kb
319	21963-L30777	Exon 8	968-969	TGGCCCGGGACC-TTGGCATGGCAG	2.0 kb
267	20206-L28038	Exon 9	1051-1052	GGACGCTCCTGG-AGCTTTGTGCCC	0.3 kb
142	20194-L27482	Exon 10	1219-1220	GCCACATTACAG-GGTGATCAGACA	
		stop codon	*1232-1234 (Exon 10)*		

Table 2d. *RAD51C* at 17q22

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_058216.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*43-45 (Exon 1)*		
202	16395-L27659	Exon 1	143-144	GGGGTTCCAGAC-TGCTGAGGAACT	2.3 kb
136	20193-L27481	Exon 2	340-341	CCCAGGGCTTCA-TAATCACCTTCT	1.7 kb
184	16393-L27657	Exon 3	578-579	CTGCATTCAGCA-CCTTCAGCTTAT	6.5 kb
301	16400-L18815	Exon 4	705-706	GAGTTACTGGCA-CAAGTTTATCTT	6.7 kb
427	20215-L27503	Exon 5	866-867	CCTTGCAAATAA-TCACAGATTAGC	10.8 kb
233	21962-L30776	Exon 6	936-935, reverse	CCTAATGCAGGA-ACAAGCAAGGCC	3.3 kb
359	20139-L27378	Exon 7	995-996	CTTTCATTGGGA-CCGAAAGCAAAG	8.4 kb
329	21964-L30778	Exon 8	1032-1031, reverse	TCCTTCTGGCTG-GGTGACTTGTAC	1.7 kb
253	16399-L28036	Exon 9	1122-1123	TTGCAAACAGAA-GGTTCCTTGAGC	
		stop codon	*1171-1173 (Exon 9)*		

[a] See section Exon numbering on page 3 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.

» Detects the same sequence as probes in SALSA MLPA Probemix P057-B2 FANCD2-PALB2.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P002 BRCA1	Contains probes for the *BRCA1* gene, involved in breast and ovarian cancer.
P087 BRCA1 Confirmation	Contains probes for the *BRCA1* gene. It can be used to confirm the results obtained with 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 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.
P057 FANCD2-PALB2	Contains probes for the *FANCD2* and *PALB2* genes, involved in Fanconi Anemia.

References

- Baker JL et al. (2015). Breast cancer in a RAD51D mutation carrier: case report and review of the literature. *Clin Breast Cancer.* 15:e71-5.
- Blanco A et al. (2012). Detection of a large rearrangement in PALB2 in Spanish breast cancer families with male breast cancer. *Breast Cancer Res Treat.* 132:307-15.
- Buys SS et al. (2017). A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. *Cancer.* 123:1721-30.
- Janatova M et al. (2013). The PALB2 gene is a strong candidate for clinical testing in BRCA1- and BRCA2-negative hereditary breast cancer. *Cancer Epidemiol Biomarkers Prev.* 22:2323-32.
- Kraus C et al. (2017). Gene panel sequencing in familial breast/ovarian cancer patients identifies multiple novel mutations also in genes others than BRCA1/2. *Int J Cancer.* 140:95-102.
- Schnurbein G et al. (2013). RAD51C deletion screening identifies a recurrent gross deletion in breast cancer and ovarian cancer families. *Breast Cancer Res.* 15:R120.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligationdependent probe amplification. Nucleic Acids Res. 30:e57.
- Susswein LR et al. (2016). Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. *Genet Med.* 18:823-32.
- Toksoy G et al. (2020). Clinical and molecular characterization of Fanconi Anemia patients in Turkey. *Mol Syndromol.* 11:183-96.
- Tung N et al. (2015). Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. *Cancer.* 121:25-33.
- Xia B at al. (2007). Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. *Nat Genet.* 39:159-61.

Selected publications using SALSA MLPA Probemix P260 PALB2-RAD50-RAD51C-RAD51D

- Borecka M et al. (2016). Mutation analysis of the PALB2 gene in unselected pancreatic cancer patients in the Czech Republic. *Cancer Genet*. 209:199-204.
- Janatova M et al. (2013). The PALB2 gene is a strong candidate for clinical testing in BRCA1- and BRCA2-negative hereditary breast cancer. *Cancer Epidemiol Biomarkers Prev*. 22:2323-32.
- Janatova M et al. (2015). Mutation analysis of the RAD51C and RAD51D genes in high-risk ovarian cancer patients and families from the Czech Republic. *PLoS One*. 10:e0127711.
- Kraus C et al. (2017). Gene panel sequencing in familial breast/ovarian cancer patients identifies multiple novel mutations also in genes others than BRCA1/2. *Int J Cancer*. 140:95-102.
- Poumpouridou N et al. (2016). Development of a novel PTT assay for mutation detection in PALB2 large exons and PALB2 screening in medullary breast cancer. *Fam Cancer*. 15:183-91.
- Sato K et al. (2017). Mutation status of RAD51C, PALB2 and BRIP1 in 100 Japanese familial breast cancer cases without BRCA1 and BRCA2 mutations. *Cancer Sci.* 108:2287-94.
- Schnurbein G et al. (2013). RAD51C deletion screening identifies a recurrent gross deletion in breast cancer and ovarian cancer families. *Breast Cancer Res*. 15:R120.
- Schubert S et al. (2019). The identification of pathogenic variants in BRCA1/2 negative, high risk, hereditary breast and/or ovarian cancer patients: High frequency of FANCM pathogenic variants. *Int J Cancer*. 144:2683-94.
- Yang C et al. (2016). Characterization of a novel germline PALB2 duplication in a hereditary breast and ovarian cancer family. *Breast Cancer Res Treat*. 160:447-56.

P260 product history	
Version	*Modification*
C1	Two probes for *RAD51C*, two probes for *RAD51D*, one probe for *PALB2* and two reference probes have been replaced.
B1	Probes for new gene *RAD51D* have been added, several target probes and reference probes have been replaced/added.
A2	Two reference probes have been replaced and probe for exon 8 of *PALB2* has been adjusted to a 3-part probe.
A1	Probes for new gene *RAD51C* have been added.
A	First release.

Implemented changes in the product description
Version C1-05 – 14 April 2022 (04P) - Sections Clinical background, including Table A, and Performance characteristics updated. - Gene structure section updated with gene sizes of *RAD51D* and *RAD50* according to UCSC Genome Browser. - Various minor textual changes. Version C1-04 – 06 April 2021 (04P) - Sample information of HG00634 corrected in the Positive samples section. - UK has been added to the list of countries in Europe that accept the CE mark. Version C1-03 – 22 January 2021 (04P) - Product description rewritten and adapted to a new template. - Intended purpose updated. - Table 1 named Table A and former Table 2 and 3 renumbered Table 1 and 2, respectively. - PMIDs in Table A replaced by references. - Gene structure and transcript variant sections updated with the most recent information. - LRG for *RAD50* added. - Links to mutation databases updated. - Two additional positive control DNA samples added. - Information about analytical performance added. - Ligation sites of the probes targeting the *PALB2*, *RAD51D* and *RAD50* genes updated according to new version of the NM_ reference sequence. Version C1-02 – 27 April 2020 (04) - Product is now registered for IVD use in Israel. Version C1-01 – 19 April 2018 (04) - Product description restructured and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 2 and Table 3). - Warning removed for 07504-L27660 probe. - Clinical background was rewritten and Table 1 was added. - A remark was added for probes with ligation sites similar or close to the probes in P057 probemix.

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