Product Description
SALSA® MS-MLPA® Probemix
ME053-A1 BRCA1-BRCA2-RAD51C

To be used with the MS-MLPA General Protocol.

Version A1.
For complete product history see page 12.

Catalogue numbers:
- **ME053-025R:** SALSA MS-MLPA Probemix ME053 BRCA1-BRCA2-RAD51C, 25 reactions.
- **ME053-050R:** SALSA MS-MLPA Probemix ME053 BRCA1-BRCA2-RAD51C, 50 reactions.
- **ME053-100R:** SALSA MS-MLPA Probemix ME053 BRCA1-BRCA2-RAD51C, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, SALSA HhaI (SMR50) 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 MS-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.

General information
The SALSA MS-MLPA Probemix ME053-A1 BRCA1-BRCA2-RAD51C is a **research use only (RUO)** assay for the detection of methylation status of one or more sequences of the *BRCA1*, *BRCA2* and *RAD51C* genes.

BRCA1, *BRCA2* and *RAD51C* are tumour suppressor genes that participate in DNA repair processes and genome integrity maintenance. Germline deleterious variants in these genes have been extensively described in hereditary breast and ovarian cancer patients, contributing to increased risks of developing the disease. Previous studies have also identified aberrant promoter methylation levels in *BRCA1* and *RAD51C* genes, proposing a new mechanism of gene inactivation in early-onset breast and/or ovarian cancer (Hansmann et al. 2012, Evans et al. 2018). Furthermore, somatic methylation of *BRCA1* and *BRCA2* may result in reduced gene expression levels and contribute to tumorigenesis and development of sporadic breast/ovarian cancer (Esteller et al. 2000, Bosviel et al. 2011, Stordal et al. 2013). Moreover, promoter hypermethylation of *BRCA1*, *BRCA2* and *RAD51C* genes has been described in triple-negative breast cancer and epithelial ovarian carcinomas, and it can guide effective therapeutic interventions with PARP inhibitors and serve as markers of therapy response (Veeck et al. 2010, Cunningham et al. 2014, Pennington et al. 2014, Sahnane et al. 2020, Menghi et al. 2022).

This SALSA MS-MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://tark.ensembl.org/

Exon numbering
The exon numbering and NM sequences used in this ME053-A1 BRCA1-BRCA2-RAD51C product description are based on the MANE Select transcripts (release version 1.0), retrieved on 02/2023. Exon numbering used here may differ from the literature. As changes to the MANE database can occur after release of this product description, exon numbering may not be up-to-date. Please note, that note that in other MRC Holland product descriptions exon numbering for the same gene might differ in case other resources are indicated to be used for exon numbering.

Probemix content
The SALSA MS-MLPA Probemix ME053-A1 BRCA1-BRCA2-RAD51C contains 38 (MS-)MLPA probes with amplification products between 123 and 400 nucleotides (nt). Ten MS-MLPA probes contain an HhaI recognition site and provide information on the methylation status of selected GCGC sites in the promoter regions of *BRCA1*, *BRCA2* and *RAD51C* genes. All probes present will also give information on copy number changes in the analysed sample. This probemix includes also 11 copy number probes flanking *BRCA1*, *BRCA2* and *RAD51C* genes. In addition, 13 reference probes are included that are not affected by HhaI digestion and target relatively copy number stable regions in various cancer types including breast and ovarian cancer. Also, three digestion control probes are included in this probemix indicating whether or not restriction endonuclease digestion in the MS-MLPA reaction was complete. Complete/partial probe sequences and the identity of the genes detected by the reference probes are available in **Table 2** and 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 MS-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)

MS-MLPA technique
The principles of the MS-MLPA technique (Nygren et al. 2005, Schouten et al. 2002) are described in the MS-MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MS-MLPA technique validation
Internal validation of the MS-MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MS-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 reference probes over the experiment.

Results of MS-MLPA are highly dependent on the HhaI enzyme used. HhaI enzymes that are resistant to heat inactivation are NOT compatible with the MS-MLPA technique and will give aberrant results. These include, but may not be limited to, Thermo Fisher Scientific enzymes HhaI, ANZA 59 HhaI, and FastDigest HhaI. We recommend using SALSA HhaI enzyme (SMR50) as this restriction enzyme has been validated for use with MS-MLPA by MRC Holland.

Required specimens
Extracted DNA, which includes DNA derived from paraffin-embedded tissues, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MS-MLPA General Protocol. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples
A sufficient number (≥3) of reference samples should be included in each MS-MLPA experiment for data normalisation and to identify the baseline methylation level for each methylation-specific probe. 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. When selecting reference samples, please note that methylation patterns may vary between tissue types and even age groups! Reference samples should be derived from different healthy individuals without a history of breast/ovarian cancer. More information regarding the selection and use of reference samples can be found in the MS-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 ACC-9, ACC-10, ACC-115, ACC-255, ACC-573, ACC-581, ACC-78 and ACC-20 from the Leibniz Institute DSMZ, Human Methylated & Non-methylated DNA Set (cat. no. D5014, Zymo Research) and Methylated Human Control DNA (cat.no. N1301, Promega) have been tested with this ME053-A1 BRCA1-BRCA2-RAD51C probemix at MRC Holland and can be used as a positive control samples to detect copy number and methylation alterations, as described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Cell line name (sample ID)	Source	Expected copy number alteration (CNA)* ◊	Expected methylation status of CpGs targeted by MS-MLPA probes in ME053-A1 #
U-266 (ACC-9)	DSMZ	heterozygous deletion of 13q12.11-q32.3 including ***BRCA2*** and flanking genes; heterozygous deletion of 17p11.2-p13.1 including *ATP1B2* and *AKAP10* genes; 17q21.33-q22 gain including ***RAD51C*** and flanking genes (*SGCA* and *COL1A1*)	none
K-562 (ACC-10)	DSMZ	heterozygous deletion of 13q12.11-q32.3 including ***BRCA2*** and flanking genes	none
MCF-7 (ACC-115)	DSMZ	***RAD51C*** high level gain	***RAD51C*** methylated
CADO-ES1 (ACC-255)	DSMZ	17q21.33-q22 gain including ***RAD51C*** and flanking genes (*SGCA* and *COL1A1*)	none
SU-DHL-8 (ACC-573)	DSMZ	***BRCA2*** gain	***RAD51C*** methylated
HCT-116 (ACC-581)	DSMZ	17q21.33-q22 gain including ***RAD51C*** and flanking genes (*SGCA* and *COL1A1*)	none
DAUDI (ACC-78)	DSMZ	none	***RAD51C*** methylated
BV-173 (ACC-20)	DSMZ	none	***RAD51C*** methylated
Human HCT116 DKO Non-Methylated DNA	Zymo Research	17q21.33-q22 gain including ***RAD51C*** and flanking genes (*SGCA* and *COL1A1*)	negative control - all targets non-methylated
Human HCT116 DKO Methylated DNA π	Zymo Research	17q21.33-q22 gain including ***RAD51C*** and flanking genes (*SGCA* and *COL1A1*)	positive control - all targets methylated
Methylated Human Control DNA π	Promega	none	positive control - all targets methylated

* Indicated chromosomal position of alteration refers to sequences targeted by (MS-)MLPA probes, however, the whole extent of CNA present in this sample cannot be determined by this ME053-A1 BRCA1-BRCA2-RAD51C probemix.
◊ CNAs detected by reference or control probes are not reported in this table.
"Methylated" refers to cases where half or more probes for the specific gene promoter have ratios higher than baseline methylation.
π Digestion control probes at 198 and 211 nt are fully digested in this methylated control sample. The HhaI digestion of these probes is NOT dependent on the methylation state of the target DNA, as the GCGC site is located in the probe's stuffer sequence.

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. Reference samples should be consulted to identify baseline methylation levels for each methylation-specific probe.

Interpretation of copy number results
The standard deviation of each individual probe over all the reference samples should be ≤0.10. When these criterion 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 / gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication / gain	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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- 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 MS-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.
- Digestion Control Probes. The target sequences of the digestion control probes are unmethylated in most blood-derived DNA samples. The signals of the digestion control probes should be gone upon complete digestion by HhaI.
- mRNA levels. We have no data showing that methylation detected by a particular probe indeed influences the corresponding mRNA levels.

- 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.

Interpretation of methylation results on blood and tissue derived DNA samples:
This probemix is intended for determining if the DNA sequences targeted by the methylation-specific probes show differential methylation as compared to the reference samples. This requires the determination of a "baseline" level of methylation, which can be used to determine if the methylation level in a test sample is significantly different from the reference samples.

The baseline methylation level must be determined for every individual methylation-specific probe, and is applicable for one particular experiment. This is important because the level of methylation in samples from healthy individuals depends on the probe's target sequence and its location in the CpG island, the tissue type and, in certain cases, even on the age of the individual. The detection of methylation can also be influenced by impurities in the DNA sample that alter the activity of the HhaI enzyme. The presence of such impurities may differ between tissue types and DNA extraction methods.

To determine the baseline methylation level, it is required to test a sufficient number (≥3) of reference samples from healthy individuals. These samples should be derived from the same tissue type, handled using the same procedure (e.g. FFPE vs. fresh frozen), and prepared using the same DNA extraction method.

The baseline methylation level is then calculated by taking the average value of final ratios of the reference samples per probe and adding two times the standard deviation. The table below contains an example. Note that each individual methylation-specific probe should have a separate baseline methylation level and those values should not be averaged between the probes.

Probe	Reference sample 1	Reference sample 2	Reference sample 3	Average	Standard deviation	Baseline level (mean+2×stdev)
Methylation-specific probe 1	0.08	0.00	0.06	0.047	0.042	0.13
Methylation-specific probe 2	0.05	0.07	0.03	0.050	0.020	0.09
Methylation-specific probe 3	0.02	0.02	0.02	0.020	0	0.02

To determine if a test sample has a significantly increased methylation level for a particular probe, compare the methylation ratio of the probe with the baseline level.

- Methylation ratio of a probe in test sample > baseline: methylation is increased.
- Methylation ratio of a probe in test sample ≤ baseline: methylation is *not* increased.

Interpretation of methylation positive samples is dependent on the application used.

NOTE: In case digestion control probes are not fully digested (>0.05), please contact info@mrcholland.com for more information.

ME053-A1 BRCA1-BRCA2-RAD51C specific notes:
- BRCA2 probe 20017-L32042 at 160 nt and BRCA2 probe 20088-L32834 at 131 nt show small residual background signal (~10-15%) after HhaI digestion. This residual signal should be taken into account for the assessment of the methylation status, as described in the paragraph above.
- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by CNAs, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Limitations of the procedure

- In most populations, the major cause of genetic defects in *BRCA1*, *BRCA2* and *RAD51C* genes are small (point) mutations, most of which will not be detected by using SALSA MS-MLPA Probemix ME053-A1 BRCA1-BRCA2-RAD51C.
- MS-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 MS-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.
- An MS-MLPA probe targets a single specific HhaI site in a CpG island; if methylation is absent for a particular CpG-site, this does not necessarily mean that the whole CpG island is unmethylated!
- Rare cases are known in which apparent methylation as detected by an MS-MLPA probe proved to be due to a sequence change in or very near the HhaI site.
- MS-MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Changes in methylation status, gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a CNA in a patient sample, especially in solid tumours with more chaotic karyotypes.

Confirmation of results

Confirmation of methylation status can be performed with another technique, such as MSP (methylation-specific PCR), pyrosequencing, digestion-based PCR assays, etc. 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 MS-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.

Cosmic mutation databases: http://cancer.sanger.ac.uk/cosmic

We strongly encourage users to deposit positive results in the COSMIC mutation database. 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 to MRC Holland: info@mrcholland.com.

Table 1. SALSA MS-MLPA Probemix ME053-A1 BRCA1-BRCA2-RAD51C

Length (nt)	SALSA MLPA probe	HhaI site	% expected signal reduction[a]	Chromosomal position (hg18)	
				Target	Reference/Control
64-105	Control fragments – see table in probemix content section for more information				
123	Reference probe 22674-L26109	-			2p13
131 ∧	**BRCA2 probe** 20088-L32834	+	85-100%	13q13.1	
139	**RAD51C probe** 22663-L32085	+	100%	17q22	
146 ¬	BLMH probe 20070-L32084	-		17q11.2	
150	**BRCA2 probe** 02285-L27214	+	100%	13q13.1	
154	Reference probe 14199-L27420	-			2q13
160 ∧ ◊	**BRCA2 probe** 20017-L32042	+	85-100%	13q13.1	
166	**BRCA1 probe** 19850-L27227	+	100%	17q21.31	
173	Reference probe 18842-L32001	-			3p14
180	**RAD51C probe** 22664-L32073	+	100%	17q22	
185 ¬	COL1A1 probe 07981-L32835	-		17q21.33	
193	Reference probe 03217-L02642	-			10q25
198 π +	**Digestion control probe** 20075-L31999	+	100%		21q22
204	Reference probe 18317-L27218	-			15q24
211 π	**Digestion control probe** 19490-L27783	+	100%		2q12
217 ∧	**BRCA2 probe** 20018-L27097	+	90-100%	13q13.1	
225	Reference probe 22675-L28369	-			19p13
230 ◊	**BRCA1 probe** 19242-L27219	+	100%	17q21.31	
238 ¬	G6PC1 probe 22617-L32087	-		17q21.31	
245	Reference probe 20072-L25185	-			10q22
252	**BRCA1 probe** 05162-L27421	+	100%	17q21.31	
260	Reference probe 22677-L27145	-			10p14
265 π	**Digestion control probe** 22931-L32871	+	100%		10q22
274	**RAD51C probe** 08852-L32873	+	100%	17q22	
283 ¬	AKAP10 probe 18257-L27424	-		17p11.2	
292	Reference probe 07154-L06766	-			19q13
301 ¬	ATP1B1 probe 22678-L26316	-		17p13.1	
310 ¬	CENPJ probe 15115-L16886	-		13q12.12	
319	Reference probe 10677-L11259	-			6p12
330 ¬	SGCA probe 11375-L32836	-		17q21.33	
337 Ж ʃ	Depurination control probe 20073-SP0891-L26870	-			16p13
346	Reference probe 17881-L22140	-			2p21
355 ¬	PCCA probe 08681-L08693	-		13q32.3	
364 ¬	ZMYM2 probe 22679-L31980	-		13q12.11	
375	Reference probe 22723-L22326	-			14q11
382 ¬	N4BP2L1 probe 22680-L01619	-		13q13.1	
391 ¬	WSB1 probe 05921-L32086	-		17q11.1	
400	Reference probe 21393-L12855	-			9q21

[a] Expected signal reduction on blood DNA derived samples. On other tissue or tumour derived samples these percentages can be different.

¬ Flanking probe: included to facilitate the determination of the extent of a deletion/duplication. CNAs of flanking and reference probes are unlikely to be related to the condition tested.

∧ This probe is not completely digested in DNA samples derived from blood.

π Digestion control: warns for insufficient digestion in MS-MLPA reaction. Upon digestion, this probe should not give a signal. Moreover, HhaI digestion of this probe is independent of the methylation status of the target DNA.

ʃ Reduced signal of this probe indicates that sample DNA is possibly depurinated. An extremely low signal of this probe might indicate a very poor sample DNA quality; please critically review your MLPA results in this case.

Ж This probe consists of three parts and has two ligation sites. This probe is included as depurination-sensitive probe: In case a sample DNA is depurinated the ratio for this probe will be lower.

+ This probe may give background signal in the digested reactions that is above the filtering threshold of Coffalyser.Net, causing a yellow warning for incomplete digestion.

◊ Target sequences of these 160 nt and 230 nt probes contain SNP rs567110692 (G/A) and rs148196794 (G/A) in the GCGC site, respectively. When the A-allele of the SNP is present, HhaI digestion will not occur, resulting in a false methylation positive signal.

NOTE: The digestion control probes at 198, 211 and 265 nt should provide no, or a very low (<10%) signal in digested samples. HhaI digestion of the 265 nt probe depends on the methylation state of the target DNA, as the GCGC site is located in the hybridizing sequence. However, the signal of 198 and 211 nt digestion control probes is NOT dependent on the methylation state of the target DNA, as the GCGC site is located in the stuffer sequence of the probe. Rare cases have been observed where the 265 nt probe generates a signal while the probes at 198 and/or 211 nt do not. In such cases, complete digestion might have been hampered by sample DNA methylation at that site, e.g. in tumour derived hypermethylated DNA or by a rare SNP in the digestion site. In these cases information obtained with 265 nt digestion control probe should be ignored. However, the digestion of this control at probe at 265 nt might be affected by impurities present in FFPE tissue-derived healthy control samples and thus positive methylation results obtained on these samples should be treated with caution.

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. ME053-A1 probes arranged according to chromosomal location

Table 2a. ME053-A1 BRCA1, BRCA2, RAD51C and flanking probes

Length (nt)	SALSA MLPA probe	Gene/ exon	Position (hg18)/ Ligation site [a]	Partial sequence (copy number probes; 24 nt adjacent to ligation site) / **Complete sequence** (MS-MLPA probes)
BRCA2 upstream flanking probes on 13q				
364 ¬	22679-L31980	*ZMYM2*	13q12.11	AGACTCTGAAAT-TCAGATTGCTAA
310 ¬	15115-L16886	*CENPJ*	13q12.12	GGTCATATTAAA-TCGTGGATTTCC
BRCA2 at 13q13.1. Indicated ligation sites are in NM_000059.4.				
217 ∧	20018-L27097	***BRCA2*** exon 1	58 nt before exon 1	CTTCCGGGTGGTGCGTGTGCTGCGTGTCGC-GTCACGGCGTCACGTGGCCAGCGCGGGCTTGT
131 ∧	20088-L32834	***BRCA2*** exon 1	6 nt before exon 1	GCGGGCTTGTGGCGCGAGCTTCTGAAACTA-GGCGGCAGAGGCGGAGCCGCTGTGGCACTGCT
160 ∧ ◊	20017-L32042	***BRCA2*** exon 1	58-59	TCTGCTGCGCCTCGGGTGTCTTTTGCG- GCGGTGGGTCG CCGCCGGGAGAAGCGTGAGGGGACAGATTTGTG
150	02285-L27214	***BRCA2*** exon 1	23 nt after exon 1	GCGGGTTAGTGGTGGTGGTAGTGGGTT-GGGACGAGCGCGTCTTCCGCAGTCCCAGTCCAGCGTGG
BRCA2 downstream flanking probes on 13q				
382 ¬	22680-L01619	*N4BP2L1*	13q13.1	CATTATTATTGA-TAATACCAACCT
355 ¬	08681-L08693	*PCCA*	13q32.3	TCCCACCAGTAA-AAGCTACCTCAA
BRCA1 upstream flanking probes on 17p and 17q				
301 ¬	22678-L26316	*ATP1B2*	17p13.1	AGAACCACCTTG-TCCTCAATTACA
283 ¬	18257-L27424	*AKAP10*	17p11.2	AGGACCAAGTCA-TGTTGCAATCAA
391 ¬	05921-L32086	*WSB1*	17q11.1	TGTCAATCCGAA-GAGTGATGCCCA
146 ¬	20070-L32084	*BLMH*	17q11.2	TCTGATACAGAA-ACTGAATTCCGA
238 ¬	22617-L32087	*G6PC1*	17q21.31	TCCTGTCAGGTA-TGGGCTGATCTG
BRCA1 at 17q21.31. Indicated ligation sites are in NM_007294.4. The location of these probes was based on the publication from Esteller et al. 2000.				
252 #	05162-L27421	***BRCA1*** exon 1	59-60	GTGGGGTTTCTCAGATAACTGGGCCCCTGC-GCTCAGGAGGCCTTCACCCTCTGCTCTGGGTAAAGGT
230 ◊	19242-L27219	***BRCA1*** exon 1	9-8 (reverse)	ATCTGAGAAACCCCACAGCCTGTCCCCCGTCCAGGAA-GTCTCAGCGAGCTCACGCCGCGCAGTCGCAGTTT
166	19850-L27227	***BRCA1*** exon 1	70 nt before exon 1	TCTGAGAGGCTGCTGCTTAGCGGTAGCCCCTT-GGTTTCCGTGGCAACGGAAAAGCGCGGGAATTACAGA
BRCA1 downstream/*RAD51C* upstream flanking probes on 17q				
330 ¬	11375-L32836	*SGCA*	17q21.33	GGACAGCTTTGA-TACCACTCGGCA
185 ¬	07981-L32835	*COL1A1*	17q21.33	CCCCAAGGACAA-GAGGCATGTCTG

***RAD51C* at 17q22. Indicated ligation sites are in NM_058216.3.**				
180	22664-L32073	***RAD51C*** upstream	106 nt before exon 1 (reverse)	GGCCTTGCAGCTTTGCGCAGTCTTGT-AAATTTTCACACTTATGCACCATTCCGTTTTGATTCCCCA
139	22663-L32085	***RAD51C*** exon 1	64-65	TGAGTCTGCGATGCGCGGGAAGACGTTCCGCT-TTGAAATGCAGCGGGATTTGGTGAGTTTCCC
274	08852-L32873	***RAD51C*** exon 1	59 nt after exon 1 (reverse)	GCTGAAGGCCGAGGCGAGAGAACGA-AGACTGAGGCGGCGCTGACGGCGGCCGGTGT

Please see **Figure 1** on page 10 for more specific information on the location of methylation-specific probes in the promoter region of *BRCA1*, *BRCA2* and *RAD51C*.

The HhaI sites are marked with grey. Ligation sites are marked with –. Complete probe sequences are available at www.mrcholland.com.

[a] Ligation sites and exon numbering are based on NM sequences from the MANE project. See section Exon numbering on page 2 for more information.

¬ Flanking probe: included to facilitate the determination of the extent of a deletion/duplication. CNAs of flanking and reference probes are unlikely to be related to the condition tested.∧ This probe is not completely digested in DNA samples derived from blood.

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.

◊ Target sequences of these 160 nt and 230 nt probes contain SNP rs567110692 (G/A) and rs148196794 (G/A) in the GCGC site, respectively. When the A-allele of the SNP is present, HhaI digestion will not occur, resulting in a false methylation positive signal.

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 2b. ME053-A1 Reference and control probes

Length (nt)	SALSA MS-MLPA probe	Gene	Chromosomal position (hg18)	Location (hg18) in kb
346	17881-L22140	*PREPL*	2p21	02-044,407
123	22674-L26109	*DYSF*	2p13	02-071,750
211 π	19490-L27783	*SLC9A2*	2q12	02-102,641
154	14199-L27420	*EDAR*	2q13	02-108,894
173	18842-L32001	*FLNB*	3p14	03-058,129
319	10677-L11259	*PKHD1*	6p12	06-052,006
400	21393-L12855	*PCSK5*	9q21	09-078,164
260	22677-L27145	*UPF2*	10p14	10-012,019
245	20072-L25185	*ANXA7*	10q22	10-074,827
265 π	22931-L32871	*ANXA7*	10q22	10-074,844
193	03217-L02642	*ADD3*	10q25	10-111,850
375	22723-L22326	*MYH7*	14q11	14-022,970
204	18317-L27218	*SEMA7A*	15q24	15-072,491
337 Ж ʃ	20073-SP0891-L26870	*GRIN2A*	16p13	16-009,831
225	22675-L28369	*CACNA1A*	19p13	19-013,289
292	07154-L06766	*DMRTC2*	19q13	19-047,047
198 π +	20075-L31999	*ITSN1*	21q22	21-034,066

Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

π Digestion control: warns for insufficient digestion. Upon digestion, this probe should not give a signal.

+ This probe may give background signal in the digested reactions that is above the filtering threshold of Coffalyser.Net, causing a yellow warning for incomplete digestion.

Ж This probe consists of three parts and has two ligation sites. This probe is included as depurination-sensitive probe: In case a sample DNA is depurinated the ratio for this probe will be lower.

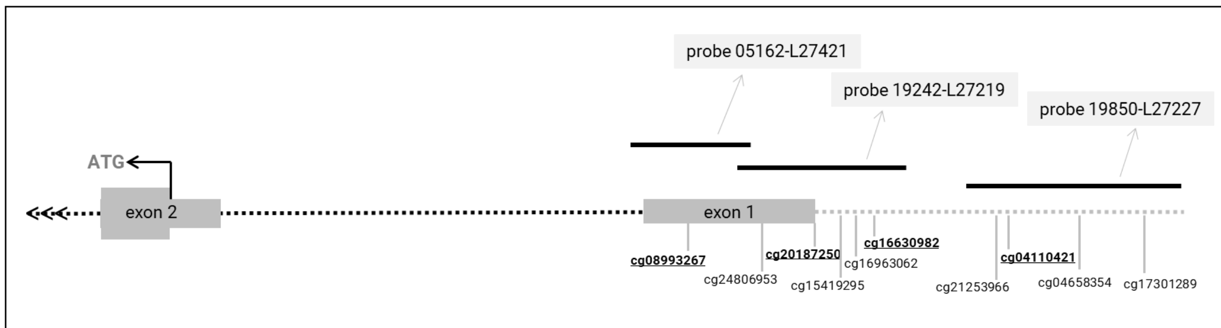
ʃ Reduced signal of this probe indicates that sample DNA is possibly depurinated. An extremely low signal of this probe might indicate a very poor sample DNA quality; please critically review your MLPA results in this case.

NOTE: The digestion control probes at 198, 211 and 265 nt should provide no, or a very low (<10%) signal in digested samples. HhaI digestion of the 265 nt probe depends on the methylation state of the target DNA, as the GCGC site is located in the hybridizing sequence. However, the signal of 198 and 211 nt digestion control probes is NOT dependent on the methylation state of the target DNA, as the GCGC site is located in the stuffer sequence of the probe. Rare cases have been observed where the 265 nt probe generates a signal while the probes at 198 and/or 211 nt do not. In such cases, complete digestion might have been hampered by sample DNA methylation at that site, e.g. in tumour derived hypermethylated DNA or by a rare SNP in the digestion site. In these cases information obtained with 265 nt digestion control probe should be ignored. However, the digestion of this control at probe at 265 nt might be affected by impurities present in FFPE tissue-derived healthy control samples and thus positive methylation results obtained on these samples should be treated with caution.

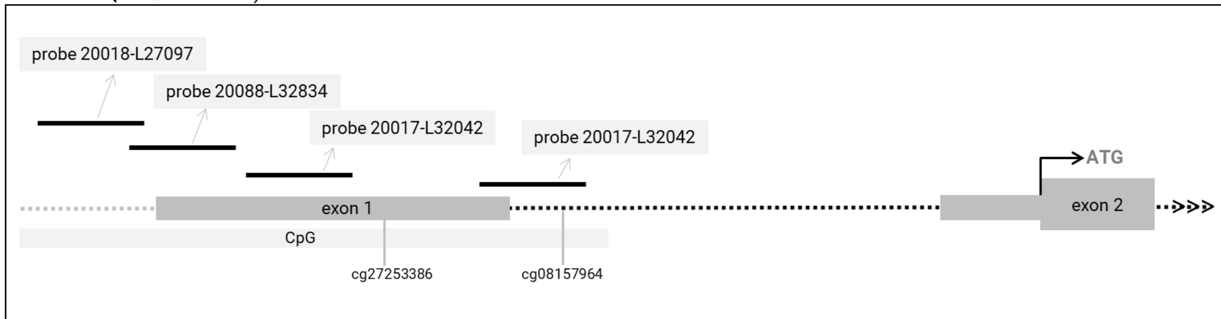
SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Figure 1. MS-MLPA probe locations in the promoter regions of *BRCA1*, *BRCA2* and *RAD51C*.

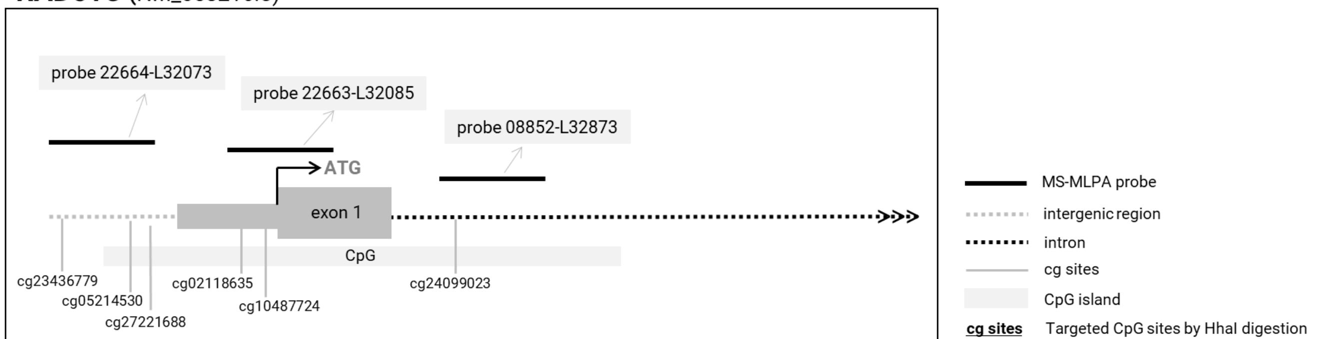
BRCA1 (NM_007294.4)



BRCA2 (NM_000059.4)



RAD51C (NM_058216.3)



Related SALSA MLPA probemixes

Application	Gene	Probemix
Breast and ovarian cancer syndrome	*BRCA1*	P002 BRCA1 P087 BRCA1 P239 BRCA1
Breast and ovarian cancer syndrome	*BRCA2*	P045 BRCA2/CHEK2 P077 BRCA2 P090 BRCA2
Breast and ovarian cancer syndrome	*RAD51C*	P260 PALB2-RAD50-RAD51C-RAD51D
Tumour suppressor genes	*BRCA1* *BRCA2*	ME001 Tumour suppressor mix

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ME053 BRCA1-BRCA2-RAD51C product history	
Version	*Modification*
A1	First release

Implemented changes in the product description
Version A1-01 – 01 March 2023 (04P) - Not applicable, new document.

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