# Product Description
## NXtec™ D001-D1 Hereditary Cancer Panel 1

To be used with the digitalMLPA NXtec Protocol.

**Version D1**
As compared to version C1, two probes targeting the *MSH2* exon 2-6 inversion have been added, one PMS2 probe has been replaced and four probes have been removed (see Table 2). The reference probe selection was adjusted and an updated set of control probes was included. Details about the added and replaced probes can be found in the probemix specific Probe Information File (PIF). For complete product history see page 12.

**Catalogue numbers**
- **D001-025R:** NXtec D001 Hereditary Cancer Panel 1, 25 reactions
- **D001-050R:** NXtec D001 Hereditary Cancer Panel 1, 50 reactions
- **D001-100R:** NXtec D001 Hereditary Cancer Panel 1, 100 reactions

NXtec D001-D1 Hereditary Cancer Panel 1 (hereafter: D001 Hereditary Cancer Panel 1) is to be used in combination with:

1. NXtec Reagent Kit (Cat No: DRK01-IL, DRK05-IL, DRK20-IL)
2. Barcode plates:
   NXtec Barcode Plate 1 (Cat No: BP01-IL (from lot 03-009-xxxxxx and higher))
   NXtec Barcode Plate 2 (Cat No: BP02-IL (from lot 03-008-xxxxxx and higher))
   NXtec Barcode Plate 3 (Cat No: BP03-IL (from lot 03-010-xxxxxx and higher))
   NXtec Barcode Plate 4 (Cat No: BP04-IL (from lot 03-011-xxxxxx and higher))

   **N.B.** The three-digit number between dashes (e.g. -008-) will increase with every new barcode plate lot.

3. Data analysis software Coffalyser digitalMLPA™ (Cat No: n.a.)

**Volumes and ingredients**

| Volumes | | | Ingredients |
|---|---|---|---|
| **D001-025R** | **D001-050R** | **D001-100R** | |
| 40 µl | 80 µl | 160 µl | Synthetic oligonucleotides, Tris-HCl, EDTA, DTT |

The probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

**Storage and handling**

| | | |
|---|---|---|
| Recommended storage conditions |   |  |

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

**Certificate of Analysis**
Information regarding quality tests is available at www.mrcholland.com.

**Precautions and warnings**
For professional use only. Always consult the most recent product description AND the digitalMLPA NXtec 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**
D001 Hereditary Cancer Panel 1 is a **research use only (RUO)** assay for the detection of deletions or duplications and the presence/absence of several mutations (including inversions) in the genes mentioned in Table 2, which are associated with hereditary predisposition for formation of breast, ovarian, colorectal, gastric, prostate, pancreatic or endometrial tumours, or for melanoma.

**This probemix is not CE/FDA registered for use in diagnostic procedures. The digitalMLPA technique is covered by US patent 6,955,901 and corresponding patents outside the US and digitalMLPA products are sold under a license of InVitae corporation on patent US 9,624,533. The purchase of this product includes a license on these patents to use only this amount of product solely for the purchaser's own use.**

**Probemix content**
A total number of 723 probes are included in D001-D1 Hereditary Cancer Panel 1, this consists of:
- 575 probes detecting copy number variations (CNVs) involved in hereditary cancer, of which three probes detect the wild-type sequence of a particular mutation. See the Probe Information File (PIF) and Table 2 for more details.
- Seven mutation-specific probes, which will only generate probe reads when that particular mutation is present (Table 2). For more information see the D001-D1 probemix-specific PIF.
- More than 120 control probes and fragments: these include probes for sample identification and probes for detection of errors or deviations when performing digitalMLPA assays, impurities in and fragmentation of the DNA samples, ligase and polymerase activity and extent of hybridisation.

The total number of probes can be used to calculate the number of reactions that can be combined into one sequencer run. See chapter "Amplicon Quantification by Illumina Sequencers" in the digitalMLPA NXtec Protocol or the calculator tool available at support.mrcholland.com.

**Reference probes**
As the target probes are spread over a large number of different autosomal chromosomal regions, no separate reference probes have been included in D001-D1 Hereditary Cancer Panel 1. Instead, a selection of 213 target probes is used as reference probes for data normalisation.

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

**digitalMLPA technique**
digitalMLPA (Benard-Slagter et al. 2017) combines the robustness and simplicity of the trusted SALSA® MLPA® technology (Schouten et al. 2002) with next-generation sequencing. For NXtec products a specific protocol of the digitalMLPA technique is used. The principles of digitalMLPA and the protocol for NXtec products are described in the digitalMLPA NXtec Protocol (www.mrcholland.com).

**digitalMLPA technique validation**
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using this NXtec probemix for the first time, or when pre-analytical steps, DNA extraction method or the instruments used are changed. This validation experiment should result in a standard deviation ≤0.10 for all probes with the exception of SNP- and mutation-specific probes.

**Required specimens**
Extracted DNA, free from impurities known to affect digitalMLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

This assay is intended for use with human genomic DNA isolated from peripheral whole blood and is not intended to be used with genomic DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials.

For more information see the digitalMLPA NXtec Protocol, section DNA sample treatment.

**Reference samples**
A sufficient number (≥3) of different reference samples from unrelated individuals should be included in each digitalMLPA 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. More information regarding the selection and use of reference samples can be found in the digitalMLPA NXtec Protocol.

When sufficient DNA samples from unrelated families are tested with D001-D1 Hereditary Cancer Panel 1, it is unlikely that the majority of the samples will have the same copy number change. In this case, using separate reference samples is not necessary and for data analysis using Coffalyser digitalMLPA the sample type should be set to "Test" (not "Reference") for all samples. The minimum number of required samples needs to be determined experimentally (read the background on our Support Portal).

However, when the testing sample set is small or includes many samples from the same family, inclusion of separate reference DNA samples in the experiment is required.

**Positive control DNA samples**
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://www.coriell.org/) and Leibniz Institute DSMZ (https://www.dsmz.de/) have a diverse collection of biological resources which may be used as a positive control DNA sample in your digitalMLPA experiments. The quality of cell lines can change, therefore deviations to the indicated CNV findings might occur. Table 1 contains a list of positive control samples that have been tested with D001 Hereditary Cancer Panel 1 at MRC Holland.

## Table 1. Positive samples from biobanks tested with D001 at MRC Holland

| Coriell sample ID | Chr. arm | Aberrant gene(s)/exons [a] | Aberration | Heterozygous/ homozygous |
|---|---|---|---|---|
| NA13451 | 2p | *EPCAM*, *MSH2* and *MSH6* | deletion | heterozygous |
| NA04127 | 3p | *MLH1* | duplication | heterozygous |
| HG00259 | 3p | *MITF* E318K (c.952G>A) mutation | present | - |
| NA11570; NA14234 | 5q | *APC* | deletion | heterozygous |
| NA07081 | 7p | *PMS2* (and *PMS2CL*) | duplication | heterozygous |
| NA02030 | 8q | *NBN* | duplication | heterozygous |
| NA03226 | 9p | *CDKN2A* | duplication | heterozygous |
| NA08618 | 11q | *ATM* | duplication | heterozygous |
| NA09596 | 11q | *ATM* | deletion | heterozygous |
| HG03694 | 11q | *ATM* exon 62-63 | duplication | heterozygous |
| NA07891 | 12q | *POLE* | duplication | heterozygous |
| | 18q | *SMAD4* | deletion | heterozygous |
| NA01535 | 12q | *POLE* | deletion | heterozygous |

| Coriell sample ID | Chr. arm | Aberrant gene(s)/exons [a] | Aberration | Heterozygous/ homozygous |
|---|---|---|---|---|
| NA02718 | 13q | *BRCA2* | deletion | heterozygous |
| NA12606 | 13q | *BRCA2* | duplication | heterozygous |
| NA03184 | 15q | *SCG5* and *GREM1* | duplication | heterozygous |
| NA20539 | 16p | *PALB2* exon 5-6 | deletion | heterozygous |
| HG03857 | 16p | *PALB2* exon 5-7 | deletion | heterozygous |
| HG00634 | 16p | *PALB2* exon 13 | duplication | heterozygous |
| NA12074 | 16q | *CDH1* | deletion | heterozygous |
| NA18949 | 17q | *BRCA1* exon 14-15 | deletion | heterozygous |
| NA14626 | 17q | *BRCA1* exon 12 | duplication | heterozygous |
| NA01359 | 18q | *SMAD4* | duplication | heterozygous |
| NA07106 | 22q | *CHEK2* | duplication | heterozygous |
| HG00187 | 22q | *CHEK2* c.1100delC mutation | present | - |

**(a) Information on the size of the deletions/duplications beyond the indicated genes can be found at https://www.coriell.org/.**

**Data analysis**

Coffalyser digitalMLPA must be used for data analysis in combination with the appropriate lot-specific product sheet. For both, the latest version should be used. Coffalyser digitalMLPA is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. Normalisation of results should be performed within one experiment. The Coffalyser digitalMLPA User Manual contains technical guidelines and information on data evaluation/normalisation.

**Interpretation of results**

Please be aware that from Coffalyser digitalMLPA 2.5 onwards in the Sample Results file only those results will be reported that are considered aberrant or that have triggered a quality warning. Consult the Excel Report for a full overview of results.

The expected results for (pseudo)autosomal probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous duplication) or ≥4 (amplification).

The standard deviation of all probes in the reference samples should be ≤0.10. When this criterion is fulfilled, the following cut-off values for the inter ratio of the probes can be used to interpret digitalMLPA results for autosomal or pseudo-autosomal chromosomes:

| Copy number status | Inter ratio |
|---|---|
| Homozygous deletion | ratio = 0 |
| Heterozygous deletion | 0.40 < ratio < 0.65 |
| Normal | 0.80 ≤ ratio ≤ 1.20 |
| Heterozygous duplication/gain | 1.30 < ratio < 1.65 |
| Heterozygous triplication/homozygous duplication/gain | 1.75 < ratio < 2.15 |
| Ambiguous copy number | All other values |

The following non-standard probes (mutation-specific, wild-type-specific and probes targeting both *PMS2* exons 12-15 and the corresponding exons in *PMS2CL*), in D001-D1 Hereditary Cancer Panel 1 require special consideration for result interpretation (see Table 2 for more information):

- Mutation-specific probes (seven probes: *MSH2*, *MITF*, *PMS2*, *CHEK2*): presence or absence will be detected with Coffalyser digitalMLPA.
- Wild-type-specific probes (*PMS2, POLE* and *BRCA2*): inter ratios for heterozygous or homozygous mutation will be detected with Coffalyser digitalMLPA.
- Probes that detect both *PMS2* exons 12-15 and the corresponding exons in *PMS2CL* (normally four copies): due to the unavailability of positive samples for each deviating copy number (two, three, five and six copies of both *PMS2* and *PMS2CL* combined) a theoretical inter ratio has been defined. In

addition, test result interpretation for exons 12-15 should be combined with test results for *PMS2*-specific probes (exons 1-11).

| Probe type | Expected inter ratios | |
|---|---|---|
| **Wild-type-specific probes (*PMS2*, *POLE*, *BRCA2*)** | normal samples | 0.80 ≤ ratio ≤ 1.20 |
| | mutant samples (heterozygous deletion or heterozygous mutation present) | 0.40 < ratio < 0.65 |
| | mutant samples (homozygous deletion, homozygous mutation, or combination of heterozygous deletion and heterozygous mutation) | ratio = 0 |
| **Probes that detect both *PMS2* exons 12-15 and the corresponding exons in *PMS2CL*** | two copies | 0.50 (0.40 < ratio < 0.65) |
| | three copies | 0.75 (0.65 < ratio < 0.85) |
| | normal samples (four copies) | 1.00 (0.85 ≤ ratio ≤ 1.15) |
| | five copies | 1.25 (1.15 < ratio < 1.35) |
| | six copies | 1.50 (1.35 < ratio < 1.65) |

General notes on digitalMLPA interpretation:

- Arranging probes according to chromosomal location facilitates interpretation of the results. 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 read count of several consecutive probes, in particular for probes located in or near a GC-rich region. The use of an alternative DNA extraction method or an additional purification step (e.g. with ethanol precipitation or silica-column-based kits) may resolve such cases. Control probes are present in all digitalMLPA probemixes that provide a warning for incomplete DNA denaturation. Sequence changes (e.g. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can also lead to false-positive results due to instable probe-DNA binding.
- False positive duplication results: Contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe read count (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: 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 digitalMLPA are pathogenic. 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. In some genes, intragenic deletions are known that result in very mild, or no disease (Schwartz et al. 2007). Duplications that include the first or last exon of a gene might in some cases not result in inactivation of that gene copy.
- Copy number changes detected by flanking probes are unlikely to have any relation to the condition tested for.

D001 Hereditary Cancer Panel 1 specific notes:

- For certain genes, such as *PMS2*, *CHEK2*, *BMPR1A* and *PTEN*, pseudogenes exist that are almost identical to the actual gene. In several cases, probes for such genes discriminate on a one nucleotide difference between gene and pseudogene. In such cases, an apparent duplication detected by a single probe can be the result of a clinically non-significant one nucleotide sequence change in one of these pseudogenes.
- For two genes, *SMAD4* and *NBN*, the presence of a processed pseudogene has been reported which is present in less than 1% of individuals tested (Mancini et al. 2015; Millson et al. 2015). These pseudogenes are probably not clinically significant and are not present yet in the human reference sequence. The presence of these pseudogenes will result in a duplication detected by some, but not all, probes for these genes.

- The D001-D1 PIF contains information on individual probes that is essential for interpretation of results.

**Limitations of the procedure**

- In most populations, the major cause of genetic defects in the genes covered by D001 Hereditary Cancer Panel 1 are small (point) mutations, most of which will not be detected by using this probemix, except for the mutations mentioned in Table 2.
- digitalMLPA cannot detect any changes that lie outside the target sequence of the probes and will detect no copy number neutral inversions or translocations except for the *MSH2* inversions mentioned in Table 2. Even when digitalMLPA does not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- **Warning:** Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results. Sequence changes can reduce the probe read count by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. See chapter 'Confirmation of results' for more information.

**Confirmation of results**

Copy number changes of multiple consecutive probes detected with D001-D1 Hereditary Cancer Panel 1 should be verified by another method when possible. MLPA probemixes are available for many genes present in D001-D1 Hereditary Cancer Panel 1. Several of these MLPA probemixes contain probes with a different ligation site that can be used for initial confirmation of results (see Table 2). Alternatively, copy number changes can be confirmed by another independent technique such as long-range PCR, qPCR, array CGH, FISH or Southern blotting.

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 result was obtained.

Please report false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com. Please contact MRC Holland for more information: info@mrcholland.com.

**Mutation database**

We strongly encourage users to deposit positive results in the Leiden Open Variation Database (lovd.nl/3.0/home). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on varnomen.hgvs.org.

## Table 2. D001-D1 Hereditary Cancer Panel 1 probe content according to chromosomal position

| Gene | Chromosomal band (hg38) | NM sequence [a] | # probes / # exons in gene | Gene length | Can be used for Confirmation [c]: Yes/No |
|---|---|---|---|---|---|
| *MUTYH* | 1p34.1 | NM_001048174.2 | 16/16 | 10.7 kb | P378 MUTYH: no<br>P072 MSH6-MUTYH: no<br>P043 APC: no |
| | **Information**: Inactivation of the *MUTYH* gene results primarily in an increased risk of colorectal and small bowel cancer (*MUTYH*-associated Polyposis syndrome; MAP). In contrast to e.g. *APC*, MAP is regarded as an autosomal recessive syndrome.<br>More information: ncbi.nlm.nih.gov/books/NBK107219/. | | | | |
| *EPCAM* | 2p21 | NM_002354.3 | 5/9 | 17.7 kb | P003 MLH1/MSH2: no<br>P072 MSH6-MUTYH: no |

| Gene | Chromosomal band (hg38) | NM sequence [a] | # probes / # exons in gene | Gene length | Can be used for Confirmation [c]: Yes/No |
|---|---|---|---|---|---|
| | **Information**: Heterozygous deletions that include the *EPCAM* transcription stop site in exon 9 are known to result in Lynch syndrome (formerly known as HNPCC) due to methylation and inactivation of the adjacent *MSH2* gene (PMID 19098912). For this reason, only the last three *EPCAM* exons are covered (by four probes). Furthermore, one probe is included that covers the 15 kb region between *EPCAM* and *MSH2* (together with four MSH2 upstream probes; see below). This probe is included only to delineate the extent of deletions/duplications.<br>More information: ncbi.nlm.nih.gov/books/NBK1211/. | | | | |
| *MSH2* | 2p21 | NM_000251.3 | 30/16 | 80.1 kb | P003 MLH1/MSH2: no<br>P248 MLH1/MSH2 confirmation: **yes** |
| | **Information**: The inactivation of one copy of the *MSH2* gene results primarily in an increased risk of colorectal and endometrial cancer (Lynch syndrome). D001 Hereditary Cancer Panel 1 includes two mutation-specific probes for the recurrent 10 Mb 2p inversion (PMIDs 24114314, 12203789 and 18335504), and two mutation-specific probes for the exon 2-6 inversion (new in version D1; PMID 26498247). Furthermore, five probes flanking the *MSH2* gene are included: one probe downstream of *MSH2* and four probes that cover the 15 kb region between *EPCAM* and *MSH2* (together with one EPCAM downstream probe; see above). These probes are included only to delineate the extent of deletions/duplications.<br>More information: ncbi.nlm.nih.gov/books/NBK1211/. | | | | |
| *MSH6* | 2p16.3 | NM_000179.3 | 19/10 | 23.8 kb | P072 MSH6-MUTYH: no |
| | **Information**: The inactivation of one copy of the *MSH6* gene results primarily in an increased risk of colorectal and endometrial cancer (Lynch syndrome). Two probes located 5-7 kb upstream of *MSH6* cover a putative regulatory region (PMID 15942939).<br>More information: ncbi.nlm.nih.gov/books/NBK1211/. | | | | |
| *BARD1* | 2q35 | NM_000465.4 | 15/11 | 84.0 kb | P489 BARD1: no |
| | **Information**: Inactivation of one copy of the *BARD1* gene results primarily in an increased risk of breast cancer. 15 probes cover all 11 *BARD1* exons and upstream region in NM_000465.4.<br>More information: PMIDs 20077502, 21344236 and 20842729. | | | | |
| *MLH1* | 3p22.2 | NM_000249.4 | 24/19 | 57.3 kb | P003 MLH1/MSH2: no<br>P248 MLH1/MSH2 confirmation: **yes** |
| | **Information**: The inactivation of one copy of the *MLH1* gene results primarily in an increased risk of colorectal, endometrial, gastric and ovarian cancer (Lynch syndrome). One probe for the *EPM2AIP1* gene upstream of *MLH1* is included only to delineate the extent of deletions/duplications.<br>More information: ncbi.nlm.nih.gov/books/NBK1211/. | | | | |
| *BAP1* | 3p21.1 | NM_004656.4 | 16/17 | 9.0 kb | P417 BAP1: no |
| | **Information**: The inactivation of one copy of the *BAP1* gene results in an increased risk of (uveal) melanomas (*BAP1* tumour predisposition syndrome).<br>More information: ncbi.nlm.nih.gov/books/NBK390611/ and PMIDs 24243779, 24187051, 23977234, 23849051 and 23684012. | | | | |
| *MITF* | 3p13 | NM_000248.4 | 1 probe | | P419 CDKN2A/2B-CDK4: no |
| | **Information**: One probe for the *MITF* gene is included. This probe is specific for the recurrent c.952G>A mutation (p.E318K; rs149617956), which has been reported as a cause for a predisposition to melanoma. The probe will only give read counts when the mutation is present.<br>More information: PMIDs 22080950, 22012259 and 24406078. | | | | |
| *APC* | 5q22.2 | NM_000038.6 | 34/16 [b] | 108.4 kb | P043 APC: **yes** |
| | **Information**: Inactivation of one copy of the *APC* gene results primarily in an increased risk of colorectal and small bowel cancer (Familial Adenomatous Polyposis; FAP). Three probes are included for the alternative exon 1 in NM_001127511.3 (PMID 25243319) and two probes for the alternative exon 2 in NM_001127510.3.<br>More information: ncbi.nlm.nih.gov/books/NBK1345/. | | | | |
| *PMS2* | 7p22.1 | NM_000535.7 | 29/15 | 38.1 kb | P008 PMS2: no |

| Gene | Chromosomal band (hg38) | NM sequence [a] | # probes / # exons in gene | Gene length | Can be used for Confirmation [c]: Yes/No |
|---|---|---|---|---|---|
| | **Information**: The inactivation of one copy of the *PMS2* gene results primarily in an increased risk of colorectal and endometrial cancer (Lynch syndrome). D001 Hereditary Cancer Panel 1 contains one probe specific for the intron 7 2-kb SVA repeat insertion as described in PMID 22461402. Also, one probe is included that is specific for the wild-type sequence at this mutation. A lower probe read count of this wild-type-specific probe can be due to either a deletion of this sequence or the insertion of the SVA element.<br>*PMS2* analysis is complicated, as there are no functional differences in exons 12, 13, 14 and 15 between *PMS2* and the corresponding exons in one of its pseudogenes, *PMS2CL*. Therefore, of the 28 copy number probes, seven probes (two probes for each exon, with the exception of exon 13) target exons 12-15 of *both PMS2* and the corresponding exons in *PMS2CL*. As each of these probes detects a sequence that is present in four (rather than two) copies per cell in unaffected individuals, a deletion or duplication of one copy will result in an inter ratio of 0.75 or 1.25, respectively, rather than the usual 0.50 or 1.50 inter ratio expected for *diploid* probe targets. For deletions/duplications affecting only exons 12-15 it is not possible to conclude where the copy number change resides based on results obtained with D001 Hereditary Cancer Panel 1: although most changes appear to be in *PMS2* itself and not in *PMS2CL* (PMID 23012243), additional experiments are required. In such cases, using SALSA MLPA Probemix P008 PMS2 alongside techniques such as long-range PCR can help reveal the location of the CNVs.<br>Please note that for several PMS2 probes, there is only one nucleotide difference between the *PMS2* sequence detected by the probe and a sequence in one of the other *PMS2* pseudogenes. In such cases, an apparent duplication detected by a single probe can be the result of a clinically insignificant one nucleotide sequence change in one of these pseudogenes.<br>More information: ncbi.nlm.nih.gov/books/NBK1211/.<br>One PMS2 probe was replaced in version D1. | | | | |
| *NBN* | 8q21.3 | NM_002485.5 | 18/16 | 51.3 kb | P494 NBN: no |
| | **Information**: Inactivation of one copy of the *NBN* gene results primarily in an increased risk of breast cancer. Please note that an *NBN* processed pseudogene might be present in a small part of the population (< 1:1000 individuals; Mancini et al., Myriad poster presented at ACMG 2015). The presence of this processed pseudogene will result in an apparent duplication of many NBN probes (this has not been tested on positive samples at MRC Holland). This processed pseudogene is not present in the human reference genome (hg38) and is probably clinically insignificant.<br>More information: ncbi.nlm.nih.gov/books/NBK1176/ and PMIDs 16770759 and 21514219. | | | | |
| *CDKN2A* | 9p21.3 | NM_000077.5 | 13/3 [b] | 7.1 kb | P419 CDKN2A/2B-CDK4: no |
| | **Information**: Inactivation of one copy of the *CDKN2A* gene results primarily in an increased risk of pancreatic cancer and melanomas. 13 probes cover the three *CDKN2A* exons in NM_000077.5 (p16INK4A) and the alternative exon 1 in NM_058195.3 (p14ARF). Two probes are present for an additional exon located between exon 2 and 3 (NM_001195132.1; p16-gamma).<br>More information: PMIDs 16234564, 10506626 and 10956390. | | | | |
| *BMPR1A* | 10q23.2 | NM_004329.3 | 17/13 | 171.4 kb | P158 JPS: **yes** |
| | **Information**: The inactivation of one copy of the *BMPR1A* gene results in an increased risk of gastric and colorectal cancer (Juvenile Polyposis Syndrome; JPS). Analysis of *BMPR1A* is complicated due to the existence of several closely related pseudogenes. Two probes are in a putative promoter region located in intron 2 (PMID 20843829). Please note that for several BMPR1A probes, there is only one nucleotide difference between the *BMPR1A* sequence detected by the probe and a sequence in one of the *BMPR1A* pseudogenes. In that case, an apparent duplication detected by a single probe can be the result of a clinically insignificant one nucleotide sequence change in one of these pseudogenes.<br>More information: ncbi.nlm.nih.gov/books/NBK1469/.<br>One *BMPR1A* probe was removed in version D1. | | | | |
| *PTEN* | 10q23.31 | NM_000314.8 | 23/9 | 108.3 kb | P225 PTEN: no<br>P158 JPS: no |

| Gene | Chromosomal band (hg38) | NM sequence [a] | # probes / # exons in gene | Gene length | Can be used for Confirmation [c]: Yes/No |
|---|---|---|---|---|---|
| | **Information**: Inactivation of one copy of the *PTEN* gene results primarily in an increased risk of breast, endometrial and thyroid cancer (PTEN Hamartoma Tumor Syndrome; PHTS). Three probes detect the single-exon *KLLN* gene which is located next to *PTEN* exon 1. Please note that for several PTEN probes, there is only one nucleotide difference between *PTEN* and the *PTEN* pseudogene. In such cases, an apparent duplication detected by a single probe could be the result of a one nucleotide sequence change in the pseudogene.<br>More information: ncbi.nlm.nih.gov/books/NBK1488/ and PMID 18972196. | | | | |
| *ATM* | 11q22.3 | NM_000051.4 | 66/63 | 146.0 kb | P041 ATM-1 & P042 ATM-2: **yes** |
| | **Information**: Inactivation of one copy of the *ATM* gene results in an increased risk of breast cancer. One probe for the *NPAT* gene upstream of *ATM* is included only to delineate the extent of deletions/duplications.<br>During validation experiments on D001 Hereditary Cancer Panel 1, we noticed two samples containing a deletion of exons 62 and 63, while in several (unrelated) samples these two exons were duplicated. Please note that duplications that include the first or last exons of a gene might not disrupt that gene and might not be clinically significant. LaBreche et al. (Myriad poster presented at ACMG 2017) indeed mention that duplication of exons 62-63 is probably not associated with an increased risk of hereditary breast cancer based on 188 observations.<br>More information: ncbi.nlm.nih.gov/books/NBK26468/ and PMIDs 16998505 and 22585167. | | | | |
| *CDK4* | 12q14.1 | NM_000075.4 | 9/8 | 4.6 kb | P419 CDKN2A/2B-CDK4: no |
| | **Information**: Inactivation of one copy of the *CDK4* gene results primarily in an increased risk of melanomas.<br>More information: PMIDs 17047042 and 10861313. | | | | |
| *POLE* | 12q24.33 | NM_006231.4 | 4/49 | 63.6 kb | P492 POLD1-POLE: no |
| | **Information**: One probe is included that is specific for the wild-type sequence at the recurrent c.1270C>G mutation (p.L424V), which has been reported as a cause for a predisposition to colorectal adenomas and carcinomas. A lower probe read count can be due to either a *POLE* deletion or the c.1270C>G mutation. During validation experiments on D001 Hereditary Cancer Panel 1, we observed a duplication of this probe in three different samples. One sample was further tested and showed a complete *POLE* gene duplication. The clinical significance of this result is not clear. To evaluate whether duplications of the wild-type probe are caused by (partial) duplications of the *POLE* gene, three additional probes are included targeting exon 2, 15 and 46.<br>More information: PMIDs 23447401, 24509466, 24501277, 25529843, 25124163 and 25370038. | | | | |
| *BRCA2* | 13q13.1 | NM_000059.4 | 42/27 | 84.8 kb | P090 BRCA2: no<br>P045 BRCA2/CHEK2: no<br>P077 BRCA2 Confirmation: **yes** |
| | **Information**: The inactivation of one copy of the *BRCA2* gene results primarily in an increased risk of breast, ovarian, prostate and pancreatic cancer (hereditary breast and ovarian cancer (HBOC) syndrome). An extra probe is included for exon 3 which is specific for the wild-type sequence at the c.156_157insAlu mutation. A lower probe read count can be due to either a deletion or the c.156_157insAlu mutation. One probe for the *ZAR1L* gene upstream of *BRCA2* is included only to delineate the extent of deletions/duplications.<br>More information: ncbi.nlm.nih.gov/books/NBK1247/. | | | | |
| *SCG5*<br>*GREM1* | 15q13.3 | NM_001144757.3<br>NM_013372.7 | 6 probes | Region covered ~68 kb | P378 MUTYH: no |
| | **Information**: A recurrent 40-kb duplication in *GREM1* has been described to result in an increased risk of colorectal cancer. Shorter duplicated regions have also been described (PMID 29804199). The presence of this duplication leads to an increased expression of the *BMP* antagonist *GREM1* and results in hereditary mixed polyposis syndrome (PMID | | | | |

| Gene | Chromosomal band (hg38) | NM sequence [a] | # probes / # exons in gene | Gene length | Can be used for Confirmation [c]: Yes/No |
|---|---|---|---|---|---|
| | 22561515). Six probes are included that are located within (five probes) or just outside (one probe) this recurrent 40-kb duplication region.<br>More information: ncbi.nlm.nih.gov/books/NBK1469/. | | | | |
| *PALB2* | 16p12.2 | NM_024675.4 | 20/13 | 38.1 kb | P260 PALB2-RAD50-RAD51C-RAD51D: **yes**<br>P057 FANCD2-PALB2: **yes** |
| | **Information**: The inactivation of one copy of the *PALB2* gene results in an increased risk of breast cancer. One probe for the *DCTN5* gene upstream of *PALB2* is included only to delineate the extent of deletions/duplications.<br>More information: PMIDs 21285249, 17287723, 17200668, 19264984 and 20412113 and ncbi.nlm.nih.gov/books/NBK1401/. | | | | |
| *CDH1* | 16q22.1 | NM_004360.5 | 20/16 | 98.2 kb | P083 CDH1: **yes** |
| | **Information**: Inactivation of one copy of the *CDH1* gene results in an increased risk of gastric and breast cancer (hereditary diffuse gastric cancer; HDGC).<br>More information: ncbi.nlm.nih.gov/books/NBK1139/. | | | | |
| *TP53* | 17p13.1 | NM_000546.6 | 14/11 [b] | 19.1 kb | P056 TP53: no |
| | **Information**: Inactivation of one copy of the *TP53* gene results in a strongly increased risk for various types of cancer (Li-Fraumeni Syndrome; LFS).<br>More information: ncbi.nlm.nih.gov/books/NBK1311/. | | | | |
| *RAD51D* | 17q12 | NM_002878.4 | 11/10 | 27.6 kb | P260 PALB2-RAD50-RAD51C-RAD51D: **yes** |
| | **Information**: Inactivation of one copy of the *RAD51D* gene results primarily in an increased risk of ovarian cancer.<br>More information: PMIDs 23372765, 22986143 and 22538716. | | | | |
| *BRCA1* | 17q21.31 | NM_007294.4 | 43/23 [b] | 81.1 kb | P002 BRCA1: no<br>P087 BRCA1 Confirmation: **yes** |
| | **Information**: The inactivation of one copy of the *BRCA1* gene results in an increased risk of breast, ovarian, prostate and pancreatic cancer (hereditary breast and ovarian cancer (HBOC) syndrome).<br>Two probes upstream of *BRCA1* are included only to delineate the extent of deletions/duplications. Deletions of exons 1 and 2 are relatively frequent (PMID 19405878). Please note that for several BRCA1 probes, there is only one nucleotide difference between the *BRCA1* gene and the *BRCA1* pseudogene. In such cases, an apparent duplication detected by a single probe could be the result of a clinically insignificant one nucleotide sequence change in the pseudogene.<br>More information: ncbi.nlm.nih.gov/books/NBK1247/. A list of publications describing the use of MLPA for *BRCA1* gene analysis can be found on the SALSA® MLPA® Probemix P002 BRCA1 product page.<br>One *BRCA1* probe was removed in version D1. | | | | |
| *RAD51C* | 17q22 | NM_058216.3 | 11/9 | 43.0 kb | P260 PALB2-RAD50-RAD51C-RAD51D: **yes** |
| | **Information**: Inactivation of one copy of the *RAD51C* gene results primarily in an increased risk of ovarian cancer. One probe for the *TEX14* gene upstream of *RAD51C* is included only to delineate the extent of deletions/duplications.<br>More information: PMIDs 20400964, 22538716, 21616938 and 20400963 and ncbi.nlm.nih.gov/books/NBK1401/. | | | | |
| *BRIP1* | 17q23.2 | NM_032043.3 | 23/20 | 184.4 kb | P240 BRIP1/CHEK1: **yes** |
| | **Information**: Inactivation of one copy of the *BRIP1* gene results in an increased risk of ovarian cancer. One probe for the *INTS2* gene upstream of *BRIP1* is included only to delineate the extent of deletions/duplications.<br>More information: ncbi.nlm.nih.gov/books/NBK1401/ and PMIDs 21964575 and 17033622. | | | | |
| *SMAD4* | 18q21.2 | NM_005359.6 | 17/12 | 54.8 kb | P158 JPS: **yes** |

| Gene | Chromosomal band (hg38) | NM sequence [a] | # probes / # exons in gene | Gene length | Can be used for Confirmation [c]: Yes/No |
|---|---|---|---|---|---|
| | **Information**: Inactivation of one copy of the *SMAD4* gene results in an increased risk of gastric and colorectal cancer (Juvenile Polyposis Syndrome; JPS; Hereditary Hemorrhagic Telangietasia; HHT). Two probes are located in the putative promoter region A (PMID 21421563) located 62 kb upstream of exon 1 (upstream of the *ELAC1* gene). Please note that a *SMAD4* processed pseudogene was described, which will result in an apparent duplication of seven SMAD4 probes (detecting exon 2, 5, 6 and 9 to 12) that are located almost entirely within exonic sequences (PMID 26165824). This has been tested on a positive sample at MRC Holland. This processed pseudogene is not present in the human reference genome (hg38) and is thought to be present in ~0.3% of the population.<br>More information: ncbi.nlm.nih.gov/books/NBK1469/. | | | | |
| *STK11* | 19p13.3 | NM_000455.5 | 15/10 | 22.7 kb | P101 STK11: **yes** |
| | **Information**: Inactivation of one copy of the *STK11* gene results in an increased risk of various types of cancer (Peutz-Jeghers syndrome). The last exon of *STK11*, which is covered by two probes, is non-coding but its presence might be required for mRNA stability. Please note that the complete *STK11* gene is located in an exceptionally GC-rich region! The presence of salt in DNA samples can hinder a complete denaturation of the *STK11* gene region, resulting in false positive deletions (or duplications when the reference samples are affected). Denaturation control probes included in the probemix can be used to detect denaturation issues in the sample.<br>More information: ncbi.nlm.nih.gov/books/NBK1266/.<br>Two *STK11* probes were removed in version D1. | | | | |
| *CHEK2* | 22q12.1 | NM_007194.4 | 21/15 | 54.1 kb | P190 CHEK2: no<br>P045 BRCA2/CHEK2: no |
| | **Information**: The inactivation of one copy of the *CHEK2* gene results in an increased risk for breast, colorectal and prostate cancer. D001 Hereditary Cancer Panel 1 contains one probe specific for the c.1100delC mutation and one probe for the *HSCB* gene upstream of *CHEK2*. This HSCB probe is included only to delineate the extent of deletions/duplications. Please note that for several CHEK2 probes, there is only one nucleotide difference between the *CHEK2* gene and *CHEK2* pseudogenes. In such cases, an apparent duplication detected by a single probe could be the result of a clinically insignificant one nucleotide sequence change in the pseudogene. The c.1100delC specific probe contains a second ligation site to be able to detect this mutation only when it is present in the *CHEK2* gene, not in its pseudogene. Homozygosity for the *CHEK2* c.1100delC mutation has been described (PMID 22058428).<br>More information: PMIDs 18172190, 15122511, 23109706, 1167536 and 17085682. | | | | |

**(a) NM sequence**: **From description version D1-01 onwards, we have adopted the MANE Select exon numbering (with the exception of *MITF*). Please note that exon numbering for the same gene might be different in other MRC Holland product descriptions, where other resources used for exon numbering are indicated.** The exon numbering and NM_ sequence used have been retrieved on 01/2024. As changes to the MANE database can occur after release of this product description, exon numbering may not be up to date. Exon numbering used here may differ from literature.
**(b) Exon numbering changed compared to the previous version of the product description.** For *APC* and *CDKN2A*, this has resulted in a different total number of exons being displayed in the table. The exon covered by each probe can be found in the PIF available at www.mrcholland.com.
**(c) Probemixes can be used for confirmation when most ligation sites are different between D001-D1 Hereditary Cancer Panel 1 probes and the probes in the corresponding probemixes.** Of note, this statement concerns the majority of the probes in a probemix and does not mean that all probes always have a different ligation site. For more information, please contact info@mrcholland.com.

More information on the location, details and warnings of the probes present in this probemix can be found in the PIF available at www.mrcholland.com.

## References


- Benard-Slagter A et al. (2017). Digital multiplex ligation-dependent probe amplification for detection of key copy number alterations in T- and B-cell lymphoblastic leukemia. *J Mol Diagn*. 19(5): 659–672.
- LaBreche H et al. (2017). Prevalence and Characterization of Triplications in Genes Associated with Hereditary Cancers. Poster 148 at Annual Clinical Genetics Meeting.

- Mancini-DiNardo D et al. (2015). Dosage Analysis by Next Generation Sequencing and Microarray CGH Indicates Putative Processed Pseudogenes in SMAD4 and NBN. Poster at Annual Clinical Genetics Meeting.
- Millson A et al. (2015). Processed Pseudogene Confounding Deletion/Duplication Assays for SMAD4. *J Mol Diagn*. 17(5):576-582.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res*. 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat*. 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem*. 421:799-801.


**Selected publications using D001 Hereditary Cancer Panel 1**


- Agiannitopoulos K et al. (2023). Copy Number Variations (CNVs) Account for 10.8% of Pathogenic Variants in Patients Referred for Hereditary Cancer Testing. *Cancer Genomics Proteomics*. 20:448-455.
- Chan SH et al. (2018). Clinical relevance of screening checklists for detecting cancer predisposition syndromes in Asian childhood tumours. *NPJ Genom Med*. 3:30.
- Chan SH et al. (2017). Germline Mutations in Cancer Predisposition Genes are Frequent in Sporadic Sarcomas. *Sci Rrep*. 7:10660.
- Rhiem K et al. (2023). Prevalence of pathogenic germline variants in women with non-familial unilateral triple-negative breast cancer. *Breast Care (Basel)*. 18:106-112.


| **D001 Hereditary Cancer Panel 1 product history** | |
|---|---|
| *Version* | *Modification* |
| D1 | Two probes targeting the *MSH2* exon 2-6 inversion added, one *PMS2* probe replaced, four probes removed, reference probe selection adjusted and an updated set of control probes included. |
| C1 | 14 target probes adjusted (not in sequence detected), two target probes replaced, one target probe removed and 19 target probes added. Reference probe selection adjusted and an updated set of control probes included. |
| B1 | First version commercially available as research use only (RUO) product. |

**Implemented changes in the product description**

Version D1-05 – 1 April 2026 (05)
- Added a remark to the Interpretation of results section about reporting of results in the Sample Results file with Coffalyser digitalMLPA 2.5 onwards.
- Updated information for *PMS2* and *PMS2CL*.
- Updated some links to extra information in Table 2.
- Various minor textual or layout changes.

Version D1-04 – 10 September (05)
- Changed sentence about Table 1: the positive control samples have been tested with D001 but not necessarily with version D1.
- Various minor textual or layout changes.

Version D1-03 – 17 July 2025 (05)
- Product names were updated throughout the document from SALSA® digitalMLPA™ to NXtec.
- 'Interpretation of results' section in the table the borders for normal samples were updated: < was replaced for ≤.
- For sample NA07081 in Table 1, the genomic aberration field was expanded with the information about a PMS2CL duplication.
- Product description adapted to a new template.

- Various minor textual or layout changes.

Version D1-02 – 05 August 2024 (04)
- The total number of probes in section Probemix Content was adjusted.

Version D1-01 – 18 July 2024 (04)
- Product description restructured and adapted to a new template.
- Changed the positive samples in Table 1.
- Product description adapted to a new product version (version number changed, changes in Table 2 and in extended information about *MSH2*).
- Restructured Table 2 (added the information and related probemixes) and removed Table 3 from the product description.
- Exon numbering of the *APC*, *BRCA1*, *CDKN2A* and *TP53* genes has been changed.
- Added related Probemix P492 POLD1 – POLE to Table 2.
- Added an extra publication to Selected publications using D001 Hereditary Cancer Panel 1.

Version C1-05 – 08 January 2024 (03)
- Replaced "SALSA digitalMLPA" with "SALSA® digitalMLPA™" where applicable.
- To be used with: section restructured and reagent kit (Cat No: DRK20-IL) added.
- Barcode plate names and lot numbers updated.
- Added sections: Ingredients, SDS note, Storage and handling, information on shelf life and safe disposal.
- Updated links to our website to https://www.mrcholland.com throughout the document.
- Added section: Selected publications using D001 Hereditary Cancer Panel 1.
- Various minor textual changes.

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