Product Description

NXtec™ D002-A1 Hereditary Cancer Panel 2

To be used with the digitalMLPA NXtec Protocol.

Version A1
First release.

Catalogue numbers

- **D002-025R:** NXtec D002 Hereditary Cancer Panel 2, 25 reactions
- **D002-050R:** NXtec D002 Hereditary Cancer Panel 2, 50 reactions
- **D002-100R:** NXtec D002 Hereditary Cancer Panel 2, 100 reactions

NXtec D002-A1 Hereditary Cancer Panel 2 (hereafter: D002 Hereditary Cancer Panel 2) 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
D002-025R	**D002-050R**	**D002-100R**	
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
NXtec D002-A1 Hereditary Cancer Panel 2 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 to cancer.

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 1172 probes are included in D002-A1 Hereditary Cancer Panel 2, this consists of:
- 1021 probes detecting copy number alterations 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.
- Ten mutation-specific probes, which will only generate probe reads when that particular mutation is present (Table 2Table 2). For more information see the D002-A1 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.

Among the 1021 probes detecting copy number alterations involved in hereditary cancer are all 575 probes of NXtec D001-D1 Hereditary Cancer Panel 1, and among the ten mutation-specific probes are all seven mutation-specific probes of NXtec D001-D1 Hereditary Cancer Panel 1.

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 D002-A1 Hereditary Cancer Panel 2. Instead, a selection of 376 target probes is used as reference probe for data normalisation.

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): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive: http://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 D002-A1 Hereditary Cancer Panel 2, 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://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your digitalMLPA experiments. The quality of cell lines can change, therefore deviations to the indicated copy number variation (CNV) findings might occur. Table 1 contains a list of positive control samples that have been tested with D002 Hereditary Cancer Panel 2 at MRC Holland.

Table 1. Positive samples from biobanks tested with D002 at MRC Holland

Coriell sample ID	Chr. arm	Aberrant gene(s)/exons [a]	Aberration	Heterozygous/ homozygous
NA00803; NA02025	1q	*SDHC*	deletion	heterozygous
NA17941	1q	*SDHC* and *FH*	duplication	heterozygous
NA06473; NA10020	1q	*FH*	deletion	heterozygous
NA05347	1q	*FH*	duplication	heterozygous
NA10401	2p	*EPCAM*, *MSH2* and *MSH6*	duplication	heterozygous
	2q	*TMEM127* and *BARD1*	duplication	heterozygous
NA13451	2p	*EPCAM*, *MSH2* and *MSH6*	deletion	heterozygous
NA10985	3p	*VHL*	deletion	heterozygous
	17q	*HOXB13* G84E (c.251G>A) mutation	present	-
NA13256	3p	*VHL*	deletion	heterozygous
NA03503; NA09552	3p	*VHL*	duplication	heterozygous
NA04127	3p	*VHL* and *MLH1*	duplication	heterozygous
HG00259	3p	*MITF* E318K (c.952G>A) mutation	present	-
NA10947	4p	*PHOX2B*	duplication	heterozygous
NA14131	5p	*SDHA*	deletion	heterozygous
NA14523	5p	*SDHA*	duplication	heterozygous
NA11570; NA14234	5q	*APC*	deletion	heterozygous

Coriell sample ID	Chr. arm	Aberrant gene(s)/exons [a]	Aberration	Heterozygous/ homozygous
NA07081	7p	*PMS2* (and *PMS2CL*)	duplication	heterozygous
NA12519	7q	*MET*	**triplication**	heterozygous
NA02030	8q	*NBN*	duplication	heterozygous
NA03226	9p	*CDKN2A*	duplication	heterozygous
NA09834	9q	*PTCH1*	deletion	heterozygous
NA08386	10q	*SUFU*	duplication	heterozygous
NA05518; NA06803	11p	*WT1*	deletion	heterozygous
NA08618	11q	*ATM*	duplication	heterozygous
NA09596	11q	*ATM*	deletion	heterozygous
HG03694	11q	*ATM* exon 62-63	duplication	heterozygous
NA15099	11q	*ATM* and *SDHD*	duplication	heterozygous
NA07891	12q	*POLE*	duplication	heterozygous
	18q	*SMAD4*	deletion	heterozygous
NA01535	12q	*POLE*	deletion	heterozygous
NA02718	13q	*BRCA2* and *RB1*	deletion	heterozygous
NA12606	13q	*BRCA2* and *RB1*	duplication	heterozygous
NA13721; NA14164	13q	*RB1*	deletion	heterozygous
NA05966	14q	*MAX*	duplication	heterozygous
NA10074	14q	*MAX* and *DICER1*	duplication	heterozygous
NA13410	14q	*DICER1*	duplication	heterozygous
NA03184	15q	*SCG5* and *GREM1*	duplication	heterozygous
NA04519	16p	*NTHL1* and *TSC2* exon 1-15	deletion	heterozygous
NA02325	16p	*NTHL1* and *TSC2*	duplication	heterozygous
	22q	*SMARCB1*, *CHEK2* and *NF2*	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
HG00180	17q	*HOXB13* G84E (c.251G>A) mutation	present	-
NA01359	18q	*SMAD4*	duplication	heterozygous
NA02571	21q	*RUNX1*	duplication	heterozygous
NA07106	22q	*SMARCB1*, *CHEK2* and *NF2*	duplication	heterozygous
HG00187	22q	*CHEK2* 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

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
Normal	0.80 ≤ ratio ≤ 1.20
Homozygous deletion	ratio = 0
Heterozygous deletion	0.40 < ratio < 0.65
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* and *PMS2CL* exons 12-15), in D002-A1 Hereditary Cancer Panel 2 require special consideration for result interpretation (see Table 2 for more information):

- Mutation-specific probes (ten probes: *MSH2*, *MITF*, *PMS2*, *FLCN*, *HOXB13*, *CHEK2*): presence or absence will be detected with Coffalyser digitalMLPA.
- Wild-type-specific probes (*PMS2, POLE* and *BRCA2*): inter ratio values for heterozygous or homozygous mutation will be detected with Coffalyser digitalMLPA.
- Probes that detect both *PMS2* and *PMS2CL* exons 12-15 (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, tests results 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 (CNV or heterozygous mutation present)	0.4 < ratio < 0.65
	mutant samples (CNV, homozygous mutation, or combination of CNV and heterozygous mutation)	ratio = 0
Probes that detect both *PMS2* and *PMS2CL* exons 12-15	normal samples (four copies)	1.00 (0.85 ≤ ratio ≤ 1.15)
	two copies	0.50 (0.40 < ratio < 0.65)
	three copies	0.75 (0.65 < ratio < 0.85)
	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: 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 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.
- Please be aware that adapter trimming should be disabled when preparing the sequencing run. (Instructions on how to prepare the run sheet are given in the digitalMLPA NXtec Protocol.) If the sequence of a probe is being trimmed, the majority of reads for this probe will disappear in all samples in the experiment. This will generate a Reference sample quality error, which fails the entire experiment. More information can be found in this support article.

D002-A1 Hereditary Cancer Panel 2 specific notes:

- For certain genes, such as *PMS2*, *CHEK2*, *BMPR1A, PTEN* and *NF1*, 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 this pseudogene will result in a duplication detected by some, but not all, probes for that gene.
- The D002-A1 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 D002 Hereditary Cancer Panel 2 are small (point) mutations, most of which will not be detected by using D002 Hereditary Cancer Panel 2, 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 D002-A1 Hereditary Cancer Panel 2 should be verified by another method when possible. MLPA probemixes are available for many genes present in D002-A1 Hereditary Cancer Panel 2. 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 (https://www.lovd.nl/3.0/home). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://varnomen.hgvs.org/.

Table 2. D002-A1 Hereditary Cancer Panel 2 probe content according to chromosomal position

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
SDHB	1p36.13	NM_003000.3	9/8	35.3 kb	P226 SDH: **yes**
	Information: Inactivation of one copy of the *SDHB* gene increases susceptibility to the development of paragangliomas and pheochromocytomas. More information: www.ncbi.nlm.nih.gov/books/NBK1548/ and PMIDs 19802898, 27485256 and 29386252.				
MUTYH	1p34.1	NM_001048174.2	16/16	10.7 kb	P378 MUTYH: no P072 MSH6-MUTYH: no 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*, *MUTYH*-associated polyposis is regarded as an autosomal recessive syndrome. More information: www.ncbi.nlm.nih.gov/books/NBK107219/.				
SDHC	1q23.3	NM_003001.5	9/6	48.8 kb	P226 SDH: **yes**
	Information: Heterozygous deletions in the *SDHC* gene are associated with an increased risk of developing paragangliomas and pheochromocytomas (Hereditary Paraganglioma/Pheochromocytoma). More information: www.ncbi.nlm.nih.gov/books/NBK1548/ and PMIDs 27485256, 19546167.				
FH	1q43	NM_000143.4	12/10	22.2 kb	P198 FH: **yes**
	Information: Inactivation of one copy of the *FH* gene results in *FH* tumour predisposition syndrome, also known as hereditary leiomyomatosis and renal cell cancer (HLRCC), which is characterized by an increased risk of developing benign leiomyomas of the skin and the uterus and/or renal cancer. One probe for the *KMO* gene upstream of *FH* is included only to delineate the extent of deletions/duplications. More information: www.ncbi.nlm.nih.gov/books/NBK1252/.				
EPCAM	2p21	NM_002354.3	5/9	17.7 kb	P003 MLH1/MSH2: no P072 MSH6-MUTYH: 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. More information: www.ncbi.nlm.nih.gov/books/NBK1211/.				
MSH2	2p21	NM_000251.3	30/16	80.1 kb	P003 MLH1/MSH2: no P248 MLH1/MSH2 confirmation: **yes**

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
	Information: The inactivation of one copy of the *MSH2* gene results primarily in an increased risk of colorectal and endometrial cancer (Lynch syndrome). D002 Hereditary Cancer Panel 2 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 (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. More information: www.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). More information: www.ncbi.nlm.nih.gov/books/NBK1211/.				
TMEM127	2q11.2	NM_017849.4	5/4	17.5 kb	P429 SDHA-MAX-TMEM127: no
	Information: Inactivation of one copy of the *TMEM127* gene is associated with an increased risk of developing pheochromocytomas and, less commonly, paragangliomas and renal cell carcinoma. More information: www.ncbi.nlm.nih.gov/books/NBK1548/ and PMIDs 33051659, 24334765.				
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. More information: PMIDs 20077502, 21344236 and 20842729.				
VHL	3p25.3	NM_000551.4	8/3	11.9 kb	P016 VHL: **yes**
	Information: Germline inactivation of one copy of the *VHL* gene predisposes individuals to Von Hippel-Lindau Disease, with tumour development typically requiring inactivation of the second gene copy in somatic cells. More information: www.ncbi.nlm.nih.gov/books/NBK1463/ and PMID 11850836.				
MLH1	3p22.2	NM_000249.4	24/19	57.3 kb	P003 MLH1/MSH2: no 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. More information: www.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). More information: www.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: Only 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. More information: PMIDs 22080950, 22012259 and 24406078.				
PHOX2B	4p13	NM_003924.4	3/3	4.6 kb	P318 Hirschsprung-2: **yes**
	Information: The inactivation of one copy of the *PHOX2B* gene is associated with an increased risk of developing neuroblastoma coinciding with neurocristopathies, among which congenital central hypoventilation syndrome. More information: omim.org/entry/613013 and www.ncbi.nlm.nih.gov/books/NBK1427/.				

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
SDHA	5p15.33	NM_004168.4	13/15	38.8 kb	P429 SDHA-MAX-TMEM127: no
	Information: The inactivation of one copy of the *SDHA* gene is associated with an increased risk of developing paraganglioma and pheochromocytoma. More information: www.ncbi.nlm.nih.gov/books/NBK1548/ and PMIDs 20484225, 21752896 and 28384794.				
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. More information: www.ncbi.nlm.nih.gov/books/NBK1345/.				
PMS2	7p22.1	NM_000535.7	29/15	38.1 kb	P008 PMS2: no
	Information: The inactivation of one copy of the *PMS2* gene results primarily in an increased risk of colorectal and endometrial cancer (Lynch syndrome). D002 Hereditary Cancer Panel 2 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. *PMS2* analysis is complicated, as there are no functional differences in exons 12, 13, 14 and 15 between *PMS2* and one of its pseudogenes. 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 its pseudogene. As each of these probes detect a sequence that is present in four (rather than two) copies per cell in normal individuals, a deletion or duplication of one copy will result in a probe ratio of 0.75 or 1.25, respectively, rather than the usual 0.5 or 1.5 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 D002 Hereditary Cancer Panel 2. Although most changes appear to be in *PMS2* itself and not in its pseudogene (PMID 23012243), additional experiments such as long range PCR or RNA analysis will be required. 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 *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. More information: www.ncbi.nlm.nih.gov/books/NBK1211/.				
MET	7q31.2	NM_000245.4	21/21	126.2 kb	P308 MET: **yes**
	Information: Germline mutations in the *MET* gene that lead to constitutive activation of the *MET* receptor are associated with an increased risk of developing papillary renal cell carcinoma. Furthermore, activating *MET* mutations are also associated to other malignancies hepatocellular carcinomas, lung cancer, breast cancer, colorectal cancer, head and neck squamous cell cancers, gastric carcinomas, and cancers of unknown origin. More information: PMIDs 31867475 and 9140397.				
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. More information: www.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				

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
	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). More information: PMIDs 16234564, 10506626 and 10956390.				
PTCH1	9q22.32	NM_000264.5	25/24	74.0 kb	P067 PTCH1: **yes**
	Information: Inactivation of one copy of the *PTCH1* gene predisposes individuals to nevoid basal cell carcinoma syndrome, increasing the risk of developing multiple basal cell carcinomas and other associated tumours. More information: www.ncbi.nlm.nih.gov/books/NBK1151/.				
TSC1	9q34.13	NM_000368.5	23/23	53.3 kb	P124 TSC1: **yes**
	Information: Inactivation of one copy of the *TSC1* gene increases the risk of developing Tuberous Sclerosis Complex. More information: www.ncbi.nlm.nih.gov/books/NBK1220/.				
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. More information: www.ncbi.nlm.nih.gov/books/NBK1469/.				
PTEN	10q23.31	NM_000314.8	23/9	108.3 kb	P225 PTEN: no P158 JPS: 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. More information: www.ncbi.nlm.nih.gov/books/NBK1488/ and PMID 18972196.				
SUFU	10q24.32	NM_016169.4	17/12	129.6 kb	P472 SUFU: **yes**
	Information: Inactivation of one copy of the *SUFU* gene results in an increased risk of developing medulloblastomas, particularly in young children, and, less commonly, basal cell carcinomas or other associated tumours. One probe for the *ACTR1A* gene upstream of *SUFU* and one probe for the *TRIM8* gene downstream of *SUFU* are included only to delineate the extent of deletions/duplications. More information: www.ncbi.nlm.nih.gov/books/NBK1151/.				
WT1	11p13	NM_024426.6	12/10	47.8 kb	P118 WT1: **yes**
	Information: Inactivation of one copy of the *WT1* gene primarily results in an increased risk of developing Wilms tumour. More information: www.ncbi.nlm.nih.gov/books/NBK556455/.				
SDHAF2	11q12.2	NM_017841.4	5/4	16.6 kb	P226 SDH: **yes**
	Information: Paternally inherited inactivation of one copy of *SDHAF2* is associated with paraganglioma, particularly those located in the head and neck region. More information: PMIDs 20071235, 26269449, 22241717, 21224366 and 28384794.				
MEN1	11q13.1	NM_001370259.2	14/10	7.0 kb	P017 MEN1: **yes** P244 AIP-MEN1-CDKN1B: **yes**
	Information: Inactivation of one copy of the *MEN1* gene results in an increased risk of developing Multiple Endocrine Neoplasia Type 1 (MEN1). More information: www.ncbi.nlm.nih.gov/books/NBK1538/.				

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
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. 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. More information: www.ncbi.nlm.nih.gov/books/NBK26468/ and at PMIDs 16998505 and 22585167.				
SDHD	11q23.1	NM_003002.4	6/4	8.9 kb	P226 SDH: **yes**
	Information: Paternally inherited heterozygous deletions in the *SDHD* gene are associated with an increased risk of developing paragangliomas, particularly in the head and neck region, and, less commonly, pheochromocytomas. More information: https://www.ncbi.nlm.nih.gov/books/NBK1548/ and PMIDs 37011647, 19802898 and 29386252.				
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. 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. 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. More information: PMIDs 23447401, 24509466, 24501277, 25529843, 25124163 and 25370038.				
BRCA2	13q13.1	NM_000059.4	42/27	84.8 kb	P090 BRCA2: no P045 BRCA2/CHEK2: no 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). 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. More information: www.ncbi.nlm.nih.gov/books/NBK1247/.				
RB1	13q14.2	NM_000321.3	29/27	178.1 kb	P047 RB1: **yes**
	Information: The inactivation of one copy of the *RB1* gene results predisposes individuals to develop retinoblastoma. More information: www.ncbi.nlm.nih.gov/books/NBK1452/.				
MAX	14q23.3	NM_002382.5	7/5	27.4 kb	P429 SDHA-MAX-TMEM127: no
	Information: Paternally inherited heterozygous deletions in the *MAX* gene are associated with an increased risk of developing pheochromocytomas and, less commonly, paragangliomas.				

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
	More information: https://www.ncbi.nlm.nih.gov/books/NBK1548/ and https://www.ncbi.nlm.nih.gov/books/NBK543228/ and PMIDs 22452945 and 21685915.				
DICER1	14q32.13	NM_177438.3	40/27	71.3 kb	P482 DICER1: **yes**
	Information: Inactivation of one copy of the *DICER1* gene results in DICER1 tumour predisposition or DICER1 syndrome, which primarily results in an increased risk for pleuropulmonary blastoma (PPB), pulmonary cysts, thyroid gland neoplasia and cystic nephroma. One probe for the *CLMN* gene upstream of *DICER1* is included only to delineate the extent of deletions/duplications. More information: https://www.ncbi.nlm.nih.gov/books/NBK196157/.				
SCG5 *GREM1*	15q13.3	NM_001144757.3 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 22561515). Six probes are included that are located within (five probes) or just outside (one probe) this recurrent 40-kb duplication region. More information: www.ncbi.nlm.nih.gov/books/NBK1469/.				
NTHL1	16p13.3	NM_002528.7	6/6	8.0 kb	-
	Information: Inactivation of both copies of the *NTHL1* gene results in an increased risk of developing *NTHL1* tumour syndrome, predisposing individuals to a wide range of cancers, particularly colorectal cancer, breast cancer, and endometrial cancer. More information: www.ncbi.nlm.nih.gov/books/NBK555473/.				
TSC2	16p13.3	NM_000548.5	41/42	41.5 kb	P046 TSC2: no P337 TSC2 Confirmation: **yes**
	Information: Inactivation of one copy of the *TSC2* gene increases the risk of developing Tuberous Sclerosis Complex. More information: www.ncbi.nlm.nih.gov/books/NBK1220/.				
PALB2	16p12.2	NM_024675.4	20/13	38.1 kb	P260 PALB2-RAD50-RAD51C-RAD51D: **yes** 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. More information: PMID 21285249, 17287723, 17200668, 19264984 and 20412113.				
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). More information: www.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). More information: www.ncbi.nlm.nih.gov/books/NBK1311/.				
FLCN	17p11.2	NM_144997.7	22/14	25.0 kb	P256 FLCN: **yes**
	Information: The inactivation of one copy of the *FLCN* gene results in an increased risk of developing fibrofolliculomas, pulmonary cysts, spontaneous pneumothorax and renal cancer. D002 Hereditary Cancer Panel 2 contains one probes specific for the c.1285delC mutation and one for the c.1285dupC mutation. These probes detect a deletion and a duplication, respectively, in a mononucleotide (C)8 tract. More information: https://www.ncbi.nlm.nih.gov/books/NBK1522/ and PMID 12204536.				
NF1	17q11.2	NM_001042492.3	65/58	282.7 kb	P081 NF1 mix 1 & P082 NF1 mix 2: **yes**

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
	Information: The inactivation of one copy of the *NF1* gene results in an increased risk of developing benign tumours, such as plexiform neurofibroma, optic pathway glioma (OPG), and non-optic central nervous system glioma. Learning disabilities are present in at least 50% of individuals with NF1. Two probes for the *RAB11FIP4* gene downstream of *NF1* are included only to delineate the extent of deletions/duplications. More information: https://www.ncbi.nlm.nih.gov/books/NBK1109/ and PMID 31370276.				
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. More information: PMIDs 23372765, 22986143 and 22538716.				
BRCA1	17q21.31	NM_007294.4	43/23 [b]	81.1 kb	P002 BRCA1: no 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). 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. More information: www.ncbi.nlm.nih.gov/books/NBK1247/. A list of more than 65 publications describing the use of MLPA for *BRCA1* gene analysis can be found in the product description of SALSA® MLPA® Probemix P002 BRCA1.				
HOXB13	17q21.32	NM_006361.6	5/2	4.0 kb	-
	Information: D002 Hereditary Cancer Panel 2 contains one probe specific for the *HOXB13* G84E (c.251G>A) mutation (rs138213197), the presence of which increases the risk of developing prostate cancer. In addition, four copy number probes are included which target *HOXB13*. More information: https://www.omim.org/entry/604607.				
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. More information: PMIDs 20400964, 22538716, 21616938 and 20400963.				
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. More information: www.ncbi.nlm.nih.gov/books/NBK1401/, PMIDs 21964575 and 17033622.				
SMAD4	18q21.2	NM_005359.6	17/12	54.8 kb	P158 JPS: **yes**
	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. More information: www.ncbi.nlm.nih.gov/books/NBK1469/.				

Gene	Chromosomal band (hg38)	NM sequence [a]	# probes / # exons in gene	Gene length	Can be used for Confirmation [b]: Yes/No
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. More information: www.ncbi.nlm.nih.gov/books/NBK1266/.				
CEBPA	19q13.11	NM_004364.5	7/1	2.6 kb	P437 Familial MDS-AML: **yes**
	Information: Inactivation of one copy of *CEBPA* gene results in an increased risk of developing *CEBPA*-associated familial acute myeloid lymphoma (AML). More information: www.ncbi.nlm.nih.gov/books/NBK47457/.				
POLD1	19q13.33	NM_002691.4	3/27	33.7 kb	P492 POLD1 - POLE: no
	Information: A germline mutation in one copy of *POLD1* gene results in an increased risk of developing Polymerase Proofreading-Associated Polyposis (PPAP), colorectal cancer, and other types of cancers, such as endometrial cancer. More information: PMIDs 32792570, 33948826, 23263490.				
RUNX1	21q22.12	NM_001754.5	14/9	261.5 kb	P437 Familial MDS-AML: **yes**
	Information: Inactivation of one copy of *RUNX1* results in an increased risk of developing hematologic malignancies. More information: www.ncbi.nlm.nih.gov/books/NBK568319/.				
SMARCB1	22q11.23	NM_003073.5	11/9	51.0 kb	P258 SMARCB1: **yes**
	Information: The inactivation of one copy of the *SMARCB1* gene results primarily in an increased risk of developing rhabdoid tumours. More information: www.ncbi.nlm.nih.gov/books/NBK469816/.				
CHEK2	22q12.1	NM_007194.4	21/15	54.1 kb	P190 CHEK2: no 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. D002 Hereditary Cancer Panel 2 contains one probe specific for the 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 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. More information: PMIDs 18172190, 15122511, 23109706, 1167536 and 17085682.				
NF2	22q12.2	NM_000268.4	17/16	95.0 kb	P044 NF2: **yes**
	Information: The inactivation of one copy of the NF2 gene results in a cancer predisposition syndrome characterized by the development vestibular schwannomas. Patients can also develop other schwannomas. More information: https://www.ncbi.nlm.nih.gov/books/NBK1201/.				

(a) NM sequence: **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 11/2024. 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) Probemixes can be used for confirmation when most ligation sites are different between D002-A1 Hereditary Cancer Panel 2 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.

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D002 Hereditary Cancer Panel 2 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-01 – 09 September 2025 (05) - Not applicable, new document.

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