digitalMLPA: Multiplex NGS-based assays to detect a wide range of structural variants implicated in hereditary cancer predisposition syndromes

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Introduction

Hereditary cancer predisposition syndromes (HCPS) account for 5-10% of all cancers. One of the most common HCPS is Lynch syndrome, which predisposes carriers of mutations in DNA mismatch repair (MMR) genes to early-onset cancer. Recently, a paracentric inversion involving MMR gene *MSH2* exon 2-6 was discovered, which may account for a subset of unexplained Lynch syndrome cases [1].

Here we present two SALSA® digitalMLPA™ probemixes that can be used to identify copy number variations (CNVs) and several single nucleotide variants (SNVs), indels and inversions in more than 50 genes linked to HCPS. In both panels, the *MSH2* exon 2-6 inversion is targeted by two probes able to detect the 3' and 5' breakpoints.

Materials and Methods

An updated version of **SALSA® digitalMLPA™ Probemix D001 Hereditary Cancer Panel 1** and an extended assay, **SALSA® digitalMLPA™ Probemix D002 Hereditary Cancer Panel 2** (hereafter D001 and D002) are in development. Included probes target the following genes:

Probemix	Genes targeted
D001 Hereditary Cancer Panel 1	*APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, POLE, PTEN, RAD51C, RAD51D, SCG5/GREM1, SMAD4, STK11, TP53*
D002 Hereditary Cancer Panel 2	**All genes included in D001 + the following:** *CEBPA, DICER1, FH, FLCN, HOXB13, MAX, MEN1, MET, NF1, NF2, NTHL1, PHOX2B, POLD1, PTCH1, RB1, RUNX1, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMARCB1, SUFU, TMEM127, TSC1, TSC2, VHL, WT1*

Additionally, several probes were included targeting the following SNVs, indels and inversions:

- *MSH2* exon 2-6 inversion [1]
- *MSH2* 10 Mb inversion [2]
- *MITF* c.952G>A
- *PMS2* SVA ins [3]
- *CHEK2* c.1100delC
- *FLCN* c.1285dupC and c.1285delC (only in D002)
- *HOXB13* G84E (only in D002)

A set of 30 blood-derived DNA samples (~40 ng DNA) with known confirmed aberrations was analysed with D001 or D002 according to the digitalMLPA general protocol (*Figure 1*). Additionally, a sample positive for the *MSH2* exon 2-6 inversion was tested. To evaluate the presence of false positives for the *MSH2* exon 2-6 inversion probes, blood-derived DNA samples from 40 healthy individuals were analysed. Sequencing was performed using the MiSeq and NextSeq 1000 Illumina platforms. No dedicated reference samples were included in any of the experiments; all test samples were normalized against the test sample population.

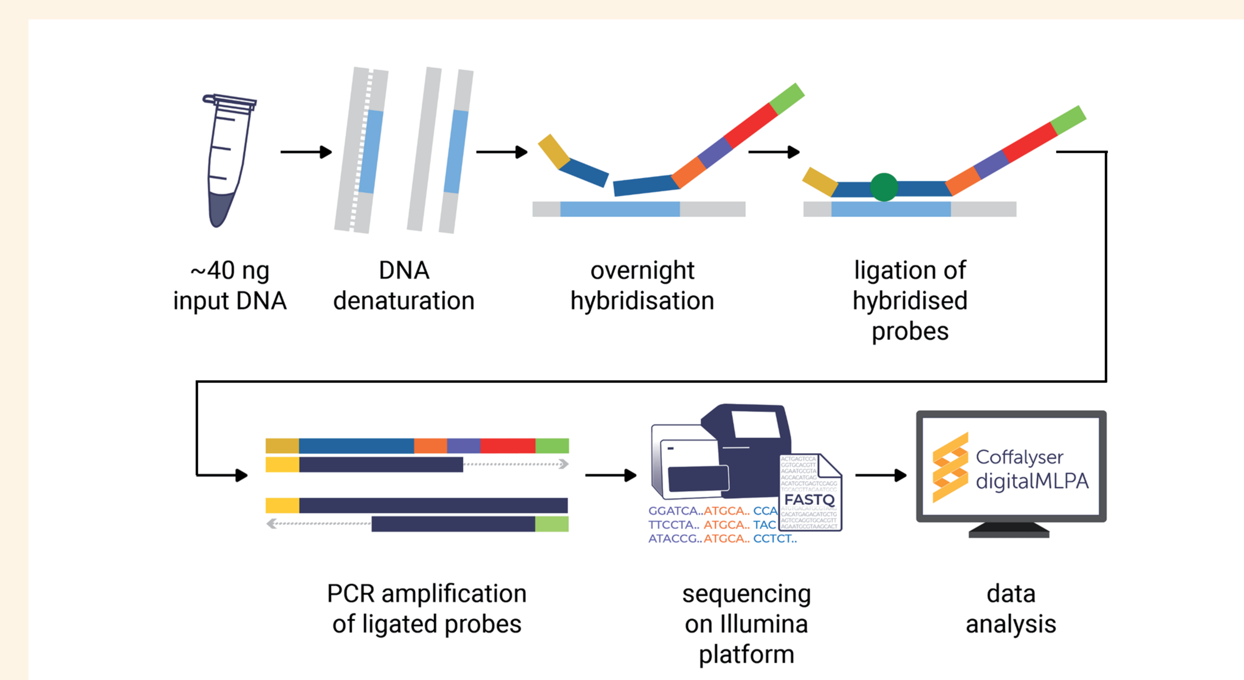

Figure 1. **digitalMLPA workflow.**

References
1. Liu Q et al. Carcinogenesis. 2016; 37:10-17.
2. Rhees J et al. Fam Cancer. 2014; 13:219-25.
3. van der Klift HM et al. Hum Mutat. 2012; 33:1051-1055

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digitalMLPA is for research use only. Not for use in diagnostic procedures.
The products described concern two test versions that are not available for general purchase.

Results

Copy number variations observed

- Whole gene deletions or duplications (selection of genes and regions: see *Figure 2*)
- Intragenic deletions or duplications (i.e., single exon duplication in *BRCA1*: see *Figure 3*)

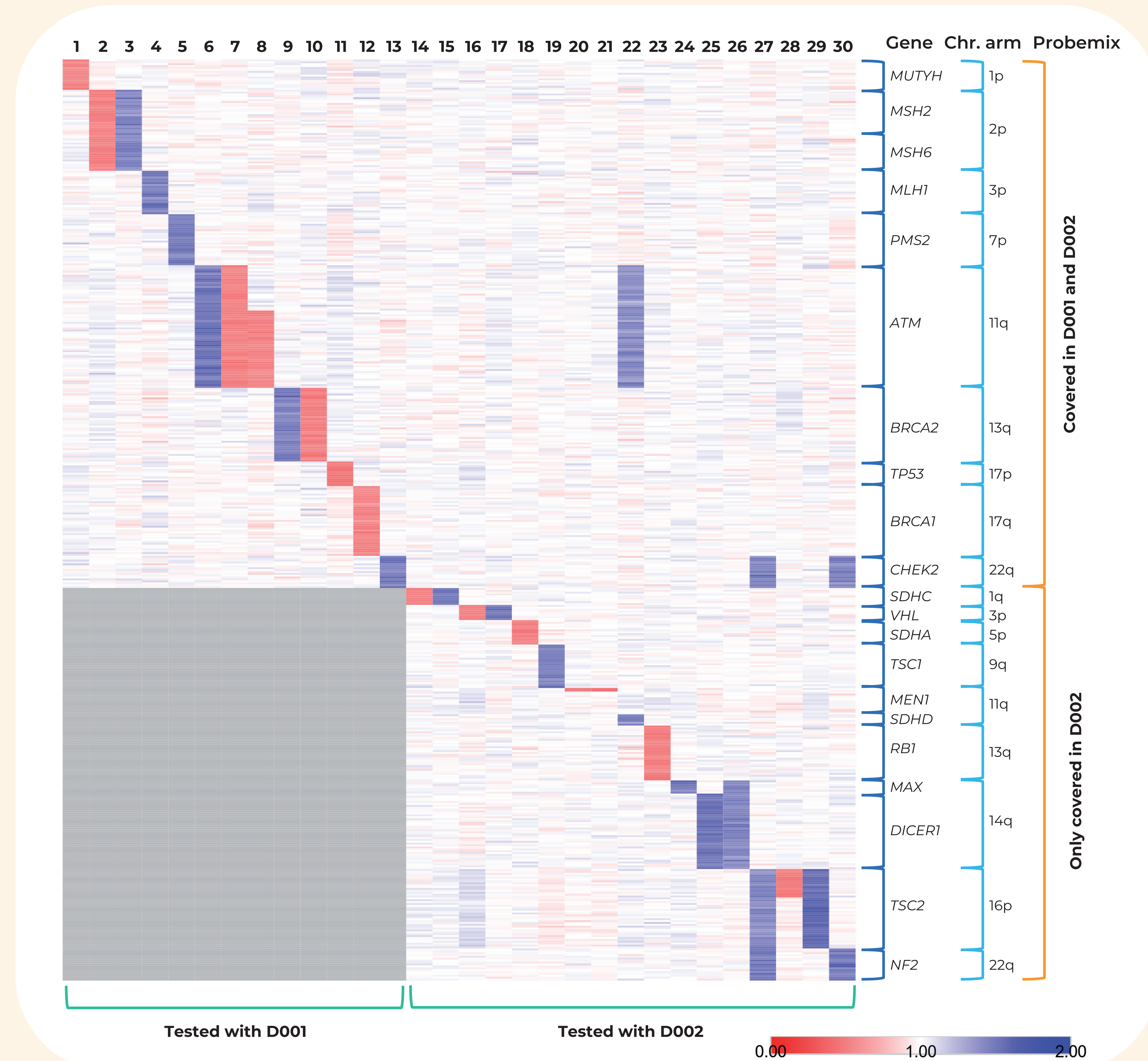

Figure 2. **Detected CNVs in selected genes.** Heat map displaying copy number (CN) ratios obtained using Coffalyser digitalMLPA™ indicating copy number status of a set of 30 samples. A subset of the genes targeted in D001 and D002 is shown. Blue indicates duplications and red indicates deletions.

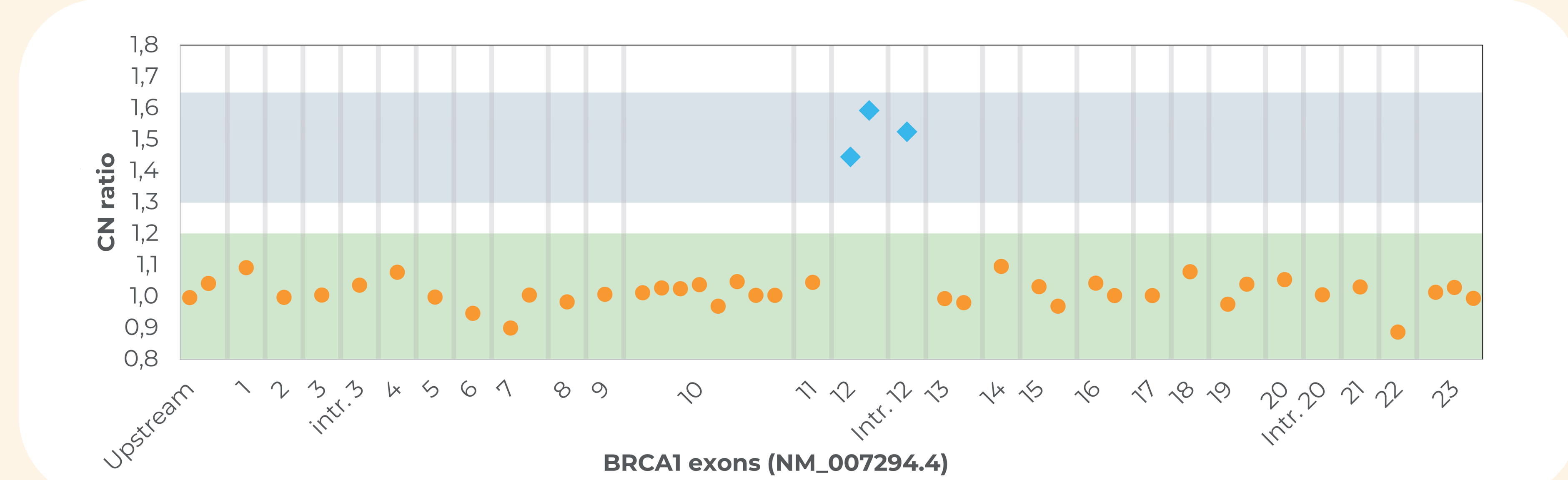

Figure 3. **CN ratio plot for a sample positive for a *BRCA1* exon 12 duplication.** Each data point represents the normalized CN ratio obtained for a single probe.

***MSH2* exon 2-6 inversion-specific probes**

Two probes were designed to detect the 3' and 5' breakpoint sequences described by Liu et al.[1]. These probes only generate a signal when the inversion is present. A schematic representation of the inversion (and binding probe location) is shown in *Figure 4A*. A sample confirmed to have the inversion by genome sequencing was tested in duplicate using D001 Hereditary Cancer Panel 1 and compared to 40 negative control samples (see *Figure 4B*).

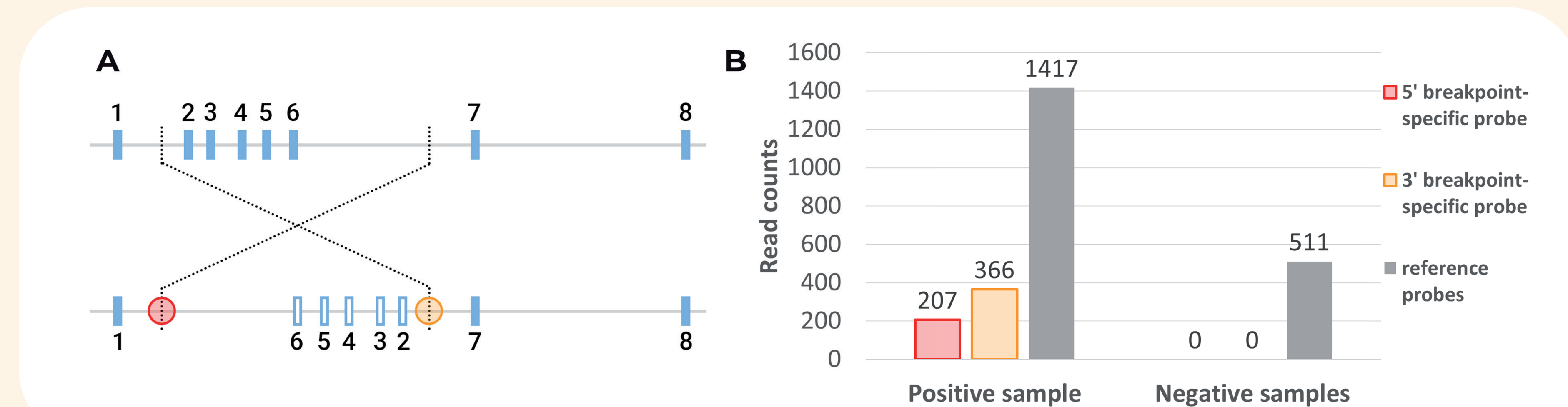

Figure 4. ***MSH2* exon 2-6 inversion. A)** Schematic representation of the *MSH2* exon 2-6 inversion (for exact details, see Liu et al.[1]). The inversion-specific probes detect the sequences indicated in red (5' breakpoint-specific) and orange (3' breakpoint-specific). **B)** Average probe read counts for the inversion-specific probes in positive and negative samples. The positive sample and negative samples were analysed in separate sequencing runs, resulting in differing absolute read counts.

Conclusions

- SALSA® digitalMLPA™ probemixes D001 Hereditary Cancer Panel 1 and D002 Hereditary Cancer Panel 2 detected CNVs from whole gene to single exon level.
- D001 Hereditary Cancer Panel 1 was able to correctly identify the *MSH2* exon 2-6 inversion. Analysis of negative control samples indicated no false positives.
- digitalMLPA can also be used to detect structural variants outside the realm of CNVs using probes targeting selected SNVs, indels and inversions.

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