HEREDITARY COLORECTAL, BREAST AND OVARIAN CANCER GENE PANEL – TECHNICAL INFORMATION V1.0

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Design: The hereditary colorectal cancer gene panel has been custom designed to include a total of 25 genes associated with hereditary colorectal, breast and ovarian cancer. The panel design enables sequencing of the full coding region and flanking intronic regions (+/- 15bp) for all of the genes in the panel with three exceptions. For *POLD1* and *POLE*, only the exonuclease proof-reading domains (exons 4-12 and 3-13, respectively) are covered. For *PMS2*, only exons 1-10 can be reliably investigated by the panel due to the pseudogene *PMS2CL* which shares significant homology with exons 11-15 of *PMS2*. These exons are analysed using long-range PCR when *PMS2* testing is indicated i.e. isolated loss of PMS2 staining in tumour tissue. For *CHEK2,* analysis is restricted to exons 1 to 9 plus the common c.1100delC variant due to the presence of a pseudogene. In addition, the 5` untranslated regions of the *APC*, *BRCA1* *BRCA2*, *MLH1*, *MSH2*, *MSH6* and *PMS2* genes are also covered by the panel. The panel has been designed to cover all biologically relevant transcripts for the genes included. Virtual subpanels (see table below) are selected during analysis to analyse the required genes for the patient.

Sub-panel	Genes included and associated sequence accession numbers
Colorectal	***APC*** (NM_000038.5), ***BMPR1A*** (NM_004329.2), ***MBD4*** (NM_003925.3), ***MLH1*** (NM_000249.3), ***MSH2*** (NM_000251.2), ***MSH3*** (NM_002439.4), ***MSH6*** (NM_000179.2), ***MUTYH*** (NM_001128425.1), ***NTHL1*** (NM_002528.5), ***PMS2*** (NM_000535.5), ***POLD1*** (NM_002691.2), ***POLE*** (NM_006231.2), ***PTEN*** (NM_000314.4), ***RNF43*** (NM_017763.5), ***SMAD4*** (NM_005359.3), ***STK11*** (NM_000455.4)
Breast	***BRCA1*** (NM_007294.3), ***BRCA2*** (NM_000059.3), ***PALB2*** (NM_024675.3), ***PTEN*** (NM_000314.4), ***STK11*** (NM_000455.4), ***TP53*** (NM_000546.5), ***ATM*** (NM_000051.3), ***CHEK2*** (NM_007194.3), ***RAD51C*** (NM_058216.2), ***RAD51D*** (NM_002878.3)
Ovarian	***BRCA1*** (NM_007294.3), ***BRCA2*** (NM_000059.3), ***BRIP1*** (NM_032043.2), ***MLH1*** (NM_000249.3), ***MSH2*** (NM_000251.2), ***MSH6*** (NM_000179.2), ***RAD51C*** (NM_058216.2), ***RAD51D*** (NM_002878.3)

Other genes: In addition to the 25 hereditary colorectal breast and ovarian cancer genes, the *CDH1* (NM_004360.3) and *DICER1* (NM_177438.2) genes are also included on the panel. These genes will only be analysed upon specific request and receipt of appropriate consent via the patient's clinical team.

Method: Library preparation and target enrichment was performed using the custom designed probe set (Twist Bioscience) and Nextera Flex for Enrichment (Illumina). Sequencing was performed using a 150bp paired-end sequencing kit on a MiSeq (Illumina). All stages of the workflow were performed according to the manufacturer's instructions.

Coverage criteria: For each sample reported, >95% of the target regions were covered to a minimum depth of 20 reads (20X). Any regions of the genes most relevant to the clinical presentation not covered at 20X depth were flagged for follow-up Sanger sequencing. These are as follows: colorectal cancer: *MLH1*, *MSH2*, *MSH6*; Lynch syndrome: *MLH1*, *MSH2*, *MSH6* (*PMS2* if isolated loss of PMS2 is indicated at

referral); Familial Adenomatous Polyposis: *APC*. Specific details of coverage and depth for individual tests are available from the laboratory on request.

Variant identification and interpretation: Sequence data were mapped and variants identified using GenomeAnalysisToolKit (GATK) and NextGENe (Softgenetics) with hg19 (GRCh37) human genome as the reference. Variants identified were subsequently classified according to the most recent ACGS Best Practice Guidelines for Variant Classification and the CanVIG-UK Consensus Specification for Cancer Susceptibility Genes of ACGS Best Practice Guidelines for Variant Classification using all available evidence. Any clinically significant variants were confirmed by Sanger sequencing.

Variant reporting: Variants were reported according to HGVS guidelines using the accession numbers listed above. Variants categorised as benign, likely benign and variants of uncertain significance not related to the phenotype were not included in the clinical report. Details of these variants are available from the laboratory on request. Please note that truncating variants only were reported for *ATM**, *CHEK2*, *MBD4* and *MSH3.*
*and *ATM* c.7271T>G p.(Val2424Gly) missense variant