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| **Patient** **PATIENT\_IN**  **URN** URN\_IN  **DOB** DOB\_IN  **Sex** SEX\_IN | **Lab No** LAB\_NO\_IN  **Ext Ref** EXT\_REF\_IN  **Collected** COLLECTED\_IN  **Received** RECEIVED\_IN  **Specimen** SPECIMEN\_IN | **Requester** REQUESTER\_IN  **Referral Lab** REFERRAL\_LAB\_IN |

**COMMENT\_IN**

**Clinical Indication** ?Germline vs somatic origin of previously detected GENE\_IN variant.

**Correlative Morphology** CORRELATIVE\_MORPHOLOGY\_IN

**Specimen Details** SPECIMEN\_DETAILS\_IN

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| **GERMLINE VARIANT ANALYSIS REPORT** |

**Test Description** Germlinevariantanalysis of all coding regions of GENE\_IN.

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| **Result Summary RESULTS\_SUMMARY1\_IN**  **Clinical Interpretation** CLINICAL\_INTERPRETATION1\_IN |

**Test Results**

**Reportable Variants**

VRF – variant read frequency

**Germline Variant Analysis:** GERMLINE\_VARIANT\_ANALYSIS\_IN

**Test Methodology**

DNA is analysed by targeted gene sequencing of coding regions and flanking splice sites (within 2 bp) of the genes listed below. Libraries are prepared using a custom QIAGEN QIAseq single primer extension-based panel (Peter MacCallum Cancer Centre AllHaem v4) and sequenced on an Illumina NextSeq500 with 150 bp paired end reads. A customised CLC bioinformatics pipeline including QIAGEN CLC enterprise solutions is used to generate aligned reads and call variants (single nucleotide variants and short insertions or deletions) against the hg19 human reference genome. Variants are analysed using PathOS software (Peter Mac) and described according to HGVS nomenclature version 19.01 (http://varnomen.hgvs.org/) with minor differences in accordance with Peter MacCallum Cancer Centre Molecular Pathology departmental policy. **Germline variant analysis** – All rare germline variants are classified according to ACMG guidelines for the interpretation of sequence variants (Richards et al. 2015, PMID: 25741868) with class 3 (uncertain significance), class 4 (likely pathogenic) and class 5 (pathogenic) variants reported only.

**Test Limitations**

The detection limit of this assay for specimens sequenced to the target read depth of 500x is a variant allele frequency (VAF) of approximately 2% (or 10% for CEBPA). This assay is primarily qualitative however, the variant read frequency (VRF) is provided to assist with variant interpretation and is assumed to approximate VAF in most instances (noting that the VAF of some insertions/deletions may be underrepresented due to assay-based allele bias). The measurement of uncertainty provided as a percentage relative standard uncertainty (i.e. CV%) for variants with VAFs of 2%, 5%-10%, 20%-40% and 50% are on average, 15.4%, 8.6%, 4.0% and 1.8%, respectively. Copy number variations, loss of heterozygosity, structural rearrangements or aneuploidies are not reported. Insertions or deletions (particularly those > 25 bp in length) are not reliably detected by this assay. Genes are analysed using the reference transcripts listed below; coding exons found in alternative transcripts are not assessed by this assay. **For germline variant analysis**, variant zygosity is assumed to be either heterozygous or homozygous in the germline based on allele frequency for the purpose of clinical interpretation. Please note Peter Mac assumes sample identification, family relationships, and clinical diagnoses are as stated on the request. Our clinical recommendations may be based on evidence from third-party data sources and should be interpreted in the context of all other clinical and laboratory information for this patient.

**Panel Summary**

Gene coverage in this sample is as follows

Please note variants may not be optimally detected in genes with less than 100% coverage. The gene coverage above is considered acceptable given the available information about the clinical context, however please contact the laboratory for further advice should specific genes covered at less than 100% require full coverage. A list of regions with suboptimal coverage is available upon request.

Please contact the laboratory on 03 8559 7284 if you wish to discuss this report further.

**Reported by REPORTED\_BY1\_IN**

**REPORTED\_BY2\_IN**

**Authorised by AUTHORISED\_BY\_IN**

**Reported 16-Nov-2023**