North West Genomic Laboratory Hub (Manchester Site)
Manchester Centre for Genomic Medicine
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NHS North West
NHS Genomic Laboratory Hub

Forename:	**GENETICSONE**	Report No:	**R24-00BK-2**
Surname:	**BEAKER**	Hospital No:	**Not provided**
Sex:	**Male**	Other No:	**Not provided**
DoB:	**01/01/1990**	Sample:	**DNA (External)**
NHS No:			
Hive Order ID:	**1000104553**		

Collected:	**Not provided**	Received:	**10/05/2024 13:27**	Activated:	**15/08/2024**
				Reported:	**04/06/2025**

Referred by: McTest Test, Wythenshawe Hospital, Manchester, M23 9LT, (test@test.com)

Genomics Laboratory Report

Reason for Testing

To investigate the cause of this individual's phenotype (see Appendix I overleaf).
Clinical indication: [Insert clinical indication title (RXXX)]. The details of any additional panels applied can be found in Appendix I overleaf.

RESULT SUMMARY:

NO PATHOGENIC VARIANTS IDENTIFIED

Result and Interpretation

Whole genome sequence analysis did not identify any pathogenic variant(s) that explain the cause of this individual's clinical presentation. This result does not exclude a genetic diagnosis.

Reported By:
Jonathan Edgerley
Clinical Scientist

Authorised By:

Jonathan Edgerley
Clinical Scientist

APPENDIX I - Test Methodology:

DNA extraction (DNA from FFPE Tissue): COBAS extraction
DNA extraction (Cell-Free DNA from Plasma): COBAS cfDNA extraction
Test Methodology: Single-primed PCR enrichment using a QIAseq Targeted DNA custom panel with Unique Molecular Identifiers (UMIs) and Illumina Next Generation Sequencing on NovaSeq6000
Genes targeted: This enrichment targets 205 commonly mutated genes (contact laboratory for further details). Please note for lung cancer patients only activating mutations in ALK, BRAF, EGFR, KRAS and MET have been reported. Re-analysis of other genes on the panel is available on request.
Bioinformatics and quality control: Mutation and variant calling by custom bioinformatic analysis pipeline validated to detect SNVs and small insertion/deletion mutations (<40bp) using Qiagen CLC software. Variants are called in the coding regions of the genes and the flanking 5bp. Variants are called down to 4% variant allele frequency (VAF) for the entire region of interest and to 2% VAF at known clinically relevant regions (hotspots).
Reference sequences: NM_004333.6 (BRAF), NM_005228.5 (EGFR), NM_004985.5 (KRAS), and NM_001127500.1 (MET).
Clinically relevant regions (hotspots): The clinically relevant regions are: EGFR codons 719, 768, 790, 851, 858 & exon 19; BRAF codons 599-600; KRAS codons 12, 13, 59-61, 117 & 147.
Coverage: Coverage is corrected for UMIs. The UMI_Depth metric can be used to statistically estimate confidence that if a variant is not observed that the variant genuinely is not present rather than that it has failed to be sampled. A UMI depth of 138x gives a 95% confidence exclusion at 4% VAF; a UMI depth of 60x gives a 95% confidence exclusion at >10% VAF. A minimum UMI depth of at least 60x was achieved across this panel including any clinically relevant codons.
Assay Sensitivity: Validation of this test has determined a sensitivity of 98.82% (95% CI: 97.27% - 99.62%) to detect variants to a 4% VAF and 99.50% (95% CI: 98.21% to 99.94%) to a 10% VAF.
Confirmation of findings: Mutations where identified are not routinely confirmed by other methods.
Limitations (FFPE Tissue): A neoplastic cell content of 20% or greater is required for optimum somatic mutation detection. Variants that occur in regions which have homology elsewhere in the genome may not be detected. Variants occurring within or adjacent to homopolymer tracts may not be detected. Other variants may have been identified that are not considered clinically relevant and/or are low confidence e.g. passenger variants in regions of low sequence coverage or variants in problematic sequence contexts (contact laboratory for details).
Limitations (Cell-Free DNA from Plasma): Variants that occur in regions which have homology elsewhere in the genome may not be detected. Variants occurring within or adjacent to homopolymer tracts may not be detected. Other variants may have been identified that are not considered clinically relevant and/or are low confidence e.g. passenger variants in regions of low sequence coverage or variants in problematic sequence contexts (contact laboratory for details).
Regulation and accreditations: This test is not UKAS accredited. The test has been locally validated and will be accredited by UKAS under the scope of ISO 15189:2012 imminently.
