NHS Lothian
South East Scotland Genetic Laboratories

OTC GENE SEQUENCING – TECHNICAL INFORMATION v.1.0

Design: *OTC* was included in a custom probe set design from Twist Bioscience. This panel design predicts coverage of 100% for the coding regions and flanking intronic sequences (+/- 15bp) for the *OTC* gene, as well as, a number of deep intronic regions encompassing previously reported pathogenic/likely pathogenic variants.

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 not covered at 20X depth were flagged for follow-up Sanger sequencing. 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 Next*GENe* (Softgenetics) with hg19 (GRCh37) human genome as the reference. Variants identified were subsequently classified according to recent 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 number listed below. Variants categorised as benign, likely benign and variants of uncertain significance that are not clinically actionable were not included in the clinical report. Details of these variants are available from the laboratory on request.

Genes included and associated sequence accession numbers:

OTC (NM_000531.5).