NHS Lothian
South East Scotland Genetic Laboratories

ALPORT SYNDROME PANEL – TECHNICAL INFORMATION

Design: The Alport syndrome gene panel was designed as part of a custom probe set (ID TE-98169478) from Twist Bioscience to cover five genes associated with Alport syndrome, thin basement membrane nephropathy and hematuria. This panel design predicts coverage of 100% for the coding regions and flanking intronic sequences (+/- 15bp) for the *COL4A1*, *COL4A3*, *COL4A4*, *COL4A5* and *MYH9* genes, as well as, a number of deep intronic regions encompassing previously reported pathogenic/likely pathogenic variants in *COL4A4* and *COL4A5*.

Method: Library preparation and target enrichment was performed using the custom designed TE-98169478 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 *COL4A3, COL4A4* and *COL4A5* genes not covered at 20X depth were flagged for follow-up Sanger sequencing if appropriate *i.e. COL4A5* fill in if clinical diagnosis of X-linked Alport syndrome, *COL4A3* or *COL4A4* fill in if one variant detected. 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 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 numbers listed below. Variants categorised as non-pathogenic, likely non-pathogenic or 'cold' VUS 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:

COL4A1 (NM_001845.6); ***COL4A3*** (NM_000091.5); ***COL4A4*** (NM_000092.5); ***COL4A5*** (NM_033380.3); ***MYH9*** (NM_002473.6).