NHS Lothian
South East Scotland Genetic Laboratories

OSTEOGENESIS IMPERFECTA GENE PANEL - TECHNICAL INFORMATION

Design: The Osteogenesis imperfecta (OI) gene panel was designed as part of a custom probe set from Twist Bioscience to cover 34 genes associated with OI. This panel design provides coverage of 100% of the target coding regions and flanking intronic sequences (+/- 15bp) for the 34 genes listed below.

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

Coverage criteria: For each sample reported, >95% of the target coding and flanking intronic regions of the 34 genes is covered to a minimum depth of 20 reads (20X). Any regions of the COL1A1 and COL1A2 genes not covered to 20X depth are 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 are mapped and variants identified using GenomeAnalysisToolKit (GATK) and NextGENe (Softgenetics) with hg19 (GRCh37) human genome as the reference. Variants identified are subsequently classified according to recent ACGS Best Practice Guidelines for Variant Classification in Rare Disease using all available evidence. Any clinically significant variants are confirmed by Sanger sequencing.

Variant reporting: Variants are reported according to HGVS guidelines using the accession numbers listed below. Variants categorised as non-pathogenic or likely non-pathogenic are filtered out at the variant interpretation stage and are not included in the clinical report. Details of these variants are available from the laboratory on request.

Genes included and associated GenBank accession numbers:

ALPL (NM_000478.6), B3GALT6 (NM_080605.4), B4GALT7 (NM_007255.3), BMP1 (NM_006129.4), CASR (NM_000388.4), COL1A1 (NM_000088.4), COL1A2 (NM_000089.4), COPB2 (NM_004766.3), CREB3L1 (NM_052854.4), CRTAP (NM_006371.5), FAM46A (TENT5A) (NM_017633.3), GORAB (NM_152281.3), FKBP10 (NM_021939.4), IFITM5 (NM_001025295.3), KDELR2 (NM_006854.4), LRP5 (NM_002335.4), MESD (NM_015154.3), NBAS (NM_015909.4), NOTCH2 (NM_024408.4), P3H1 (LEPRE1) (NM_022356.4), P4HB (NM_000918.4), PLOD2 (NM_182943.3), PLS3 (NM_005032.7), PPIB (NM_000942.5), SEC24D (NM_014822.4), SERPINF1 (NM_002615.7), SERPINH1 (NM_001235.5), SP7 (NM_001173467.3), SPARC (NM_003118.4), TAPT1 (NM_153365.3), TMEM38B (NM_018112.3), TRPV6 (NM_018646.6), UNC45A (NM_018671.5), WNT1 (NM_005430.4).