NHS Lothian
South East Scotland Genetic Laboratories

Primordial Dwarfism and Microcephaly Panel- Technical information

Design: The Primordial Dwarfism and Microcephaly panel was designed as a custom probe set (Twist Bioscience) and includes the genes listed below. This panel predicts coverage of >98% of all coding exons as reported on RefSeq and their immediate intronic sequences (+/-15bp).

Method: Library preparation is carried out using the Illumina DNA Prep with Enrichment reagent kit. Target enrichment is performed using the custom-designed baits (Twist Bioscience), followed by paired-end sequencing on the MiSeq platform (Illumina). All stages of the workflow were carried out according to the manufacturer's instructions (Illumina).

Sequence quality: For each sample reported, >95% of the coding sequences and flanking intronic sequences of the 57 genes were covered to a minimum depth of 20 reads. Depending on the referral, any regions of the *ASPM* and *PCNT* genes not covered at 20X depth are flagged for follow-up Sanger sequencing, (regions of ASPM are filled if OFC is -4SD or smaller, regions of PCNT are filled if OFC -5SD or smaller AND height is -5SD or smaller). More 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/cool 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:

ANKRD11 (NM_001256183.1), **ASPM** (NM_018136.4), **ATR** (NM_001184.3), **ATRX** (NM_000489.3), **BLM** (NM_000057.2), **CASK** (NM_003688.3), **CDC45** (NM_001178010.2), **CDC6** (NM_001254.3), **CDKN1C** (NM_000076.2), **CDK5RAP2** (NM_018249.5), **CDT1** (NM_030928.3), **CENPF** (NM_016343.3), **CENPJ** (NM_018451.4), **CEP135** (NM_025009.4), **CEP152** (NM_001194998.1), **CEP63** (NM_025180.3), **CREBBP** (NM_004380.2), **DNA2** (NM_001080449.2), **DNMT3A** (NM_175629.2) (PWWP domain only), **DONSON (**NM_017613.3), **DPP6** (NM_130797.3), **DYRK1A** (NM_001396.3), **EP300** (NM_001429.3), **GMNN** (NM_015895.4), **IGF1** (NM_000618.4), **IGF1R** (NM_000875.4), **KIF11** (NM_004523.3), **KMT2A (**NM_001197104.1), **KNL1** (NM_170589.4), **LARP7** (NM_001267039.1), **LIG4** (NM_002312.3), **MCPH1** (NM_001322042.1), **MRE11** (NM_005591.3), **NBN** (NM_002485.4), **NDE1** (NM_001143979.1), **ORC1** (NM_004153.3), **ORC4** (NM_001190879.2), **ORC6** (NM_014321.3), **PCNT** (NM_006031.5), **PLK4** (NM_014264.4), **PNKP (**NM_007254.4), **POC1A** (NM_015426.4), **POLE (**NM_006231.3), **RAD50** (NM_005732.3), **RBBP8** (NM_002894.2), **RNU4ATAC** (NR_023343.1), **SMARCAL1** (NM_014140.3), **SRCAP** (NM_006662.2), **STIL** (NM_001048166.1), **TCF4** (NM_001243226.2), **TOP3A (**NM_004618.4), **TRAIP** (NM_005879.2), **TUBGCP6** (NM_020461.3), **VPS13B** (NM_017890.4), **WDR4** (NM_033661.4), **WDR62** (NM_001083961.1), **XRCC4** (NM_022406.3/NM_001318012.1).